NUTRITIONAL QUALITY, MICROSTRUCTURAL PROPERTIES AND CONSUMER ACCEPTABILITY OF BISCUITS OBTAINED FROM FERMENTED FINGER MILLET (*ELEUSINE CORACANA*) FLOURS.

by

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DECLARATION

I, Mudau Masala, Student No. 11632442, hereby declare that this Master of Science in Food Science and Technology dissertation, which I have submitted to the Department of Food Science and Technology under the Faculty of Science, Engineering, and Agriculture at the University of Venda, is my work, which I conducted under the supervision of Dr. S.E. Ramashia and Mr. M.E. Mashau. No other researchers have ever submitted this work for a degree at any university. All the information sources used in this study have been acknowledged.

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ABSTRACT

Finger millet (FM) (*Eleusine coracana*) is one of the most important minor cereal grains. Its fibre and ash contents are higher than that of major cereals such as wheat and rice. The grains are gluten-free therefore, they should be incorporated in the diet of celiac patients. The objective of this study was to determine the nutritional quality, microstructural properties, and consumer acceptability of biscuits prepared using spontaneously fermented FM flours. The FM grains were fermented at Different period (24, 48 & 72 h) of fermentation. The mineral content of FM flours used in biscuits making, as well as their functional, thermo-pasting, and microstructural properties were investigated. Biscuits were subjected to nutritional quality analyses (proximate composition & minerals), antioxidant, microstructural and sensory properties analyses. The results obtained showed that spontaneous fermentation (SF) decreased the packed bulk density (PBD), swelling capacity (SC), and increased water absorption capacity (WAC), and oil absorption capacity (OAC) of FM flours. The loose bulk density (LBD) values showed that SF had no effect, as no significant differences ($p < 0.05$) were noted. The LBD, PBD, WAC, OAC and SC of FM flours were within the range of 0.47 to 0.56 g/g, 0.71 to 0.79 g/g, 1.96 to 2.14 g/g, 1.24 to 1.44 g/g, and 13.33 to 14.33 mL, respectively. The cooked paste viscosity of FM flours decreased with increasing period of SF, ranging from 253.67 to 421.67 cP. The process of SF also induced changes in the thermal properties of FM flours as an increment of gelatinization temperatures and gelatinisation enthalpy with increasing time of SF was observed. The onset, peak, and conclusion temperature of FM flours ranged from 69.42 to 82.47 °C, 70.98 to 84.48 °C, and 78.24 to 86.39 °C, respectively. The gelatinisation temperature range and gelatinisation enthalpy of FM flours were within the range of 3.01 to 9.59 °C and 2.99 to 5.07 J/g, respectively. The reduction in the peak viscosity (PV), trough viscosity (TV), and final viscosity (FV) and increment in the breakdown viscosity (BDV), setback viscosity (SV), peak time (Pt), peak temperature (PT) of fermented FM flours were observed. The PV, TV, BDV, FV, SV, PT, and Pt ranged from 1709.67 to 2876.67 cP, 1349.67 to 2739.7 cP, 102.00 to 360.00 cP, 1616.33 to 2959.00 cP, 105.33 to 349.33 cP, 5.11 to 6.80 min, and 74.82 to 75.73°C, respectively. The enhancement in the mineral compositions of FM flours as SF time increased was noted. The microstructural changes in the starch granules as SF time increased were also observed. As for the biscuits, the decrease in moisture, ash, crude fiber, and crude fat contents as well as total phenolic and flavonoids contents as SF time increased was observed. Protein content, carbohydrates content, antioxidant activity, and mineral compositions of FM biscuits increased as SF time increased. The colour attributes such as lightness (L*), showed a significant increase as fermentation time increased in light brown FM biscuits, however dark brown FM biscuits showed no significant differences ($p < 0.05$). The hue angle and total colour differences (ΔE) of FM biscuits increased with increasing time of SF. The reduction in the physical properties of FM biscuits including diameter, weight, spread ratio and an increment in thickness and texture as SF time increased were also observed. The diameter, thickness, weight, spread ratio and hardness of FM biscuits were within the range of 4.54 to 4.76 cm, 0.66 to 0.76 cm, 11.99 to 12.77 g, 6.05 to 7.25 and 689.61 to 2372.23 g, respectively. Spontaneous fermentation also induced changes on the microstructure of FM biscuits. Among the fermented FM biscuits, panellists preferred 24 h gluten-free fermented FM biscuits since they had better sensory properties. Overall, SF enhanced the functional properties, thermo-pasting properties, mineral compositions, and nutritional value of FM flours, as well as the potential health benefits of eating gluten-free FM biscuits.

Key words: Millet, Spontaneous fermentation, Gluten-free, Nutritional properties, Microstructural properties, Sensory evaluation.

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LIST OF ACRONYMS

a*	Redness
$AlCl_3$	Aluminium chloride
ANFs	Antinutritional factors
ANOVA	Analysis of variance
b*	Yellowness
BDV	Breakdown viscosity
Ca	Calcium
QE	Quercetin equivalent
Cu	Copper
CW	Cell wall
DBFMB	Dark brown finger millet biscuit
DBFMF	Dark brown finger millet flour
DPPH	2,2-diphenyl-lpicrylhydrazyl
DSC	Differential Scanning Calorimeter
FB	Fermented biscuits
Fe	Iron
FM	Finger millet
FMF	Finger millet flour
Frap	Ferric reducing antioxidant power
FTIR	Fourier transform-infrared
FV	Final viscosity
GAE	Gallic acid equivalent
H°	Hue angle
ICP-AEC	Inductively coupled argon plasma atomic emission
$K_3[Fe\ (CN)_6]$	Potassium ferricyanide
K	Potassium
GF	Gluten-free
LBFMB	Light brown finger millet biscuit
LBFMF	Light brown finger millet flour
L*	Lightness
LBD	Loose bulk density
LBFMF	Light brown finger millet flour
Mg	Magnesium
Mn	Manganese
Na	Sodium
NaOH	Sodium hydroxide

$NaNO_2$	Sodium nitrite
NB	Native biscuits
OAC	Oil absorption capacity
P	Phosphorus
PB	Protein bodies
PBD	Packed bulk density
PLM	Protein lipid matrix
Pt	Peak time
PT	Peak temperature
PV	Peak viscosity
RTE	Ready-to-eat
SC	Swelling capacity
SEM	Scanning electron microscopy
SF	Spontaneous fermentation
SG	Starch granules
SV	Setback viscosity
TFC	Total flavonoid contents
Tc	Conclusion temperature
To	Onset temperature
Tp	Peak temperature
TPC	Total phenolic contents
Tr	Temperature range
TV	Trough viscosity
XRD	X- ray diffraction
Zn	Zinc
ΔE	Total colour difference

LIST OF SI UNITS

°C	Degree celsius
G	Gram
H	Hour(s)
J/g	Joule per gram
Kcal	kilocalories
µL	Microliter
µm	Micrometre
Mg	Milligram
mL	Millilitre
M	Molar
Min	Minute(s)
Nm	Nanometre
%	Percentage

DISSERTATION OUTLINE

This dissertation consists of five (5) chapters as listed below:

CHAPTER ONE: INTRODUCTION

This chapter provides some background on millets in general and finger millet cereal grains specifically. It also presents the problem that needs to be addressed. The justification, aim, specific objectives and hypotheses of the study are in this chapter.

CHAPTER TWO: LITERATURE REVIEW

Chapter two (2) provides a comprehensive overview of finger millet, including its origins, structure, components, health advantages, application, and processing prior to ingestion. It also discusses the beneficial effects of fermentation on cereal grains especially finger millet.

CHAPTER THREE: MINERAL CONTENT, FUNCTIONAL, THERMO-PASTING, AND MICROSTRUCTURAL PROPERTIES OF SPONTANEOUSLY FERMENTED FINGER MILLET FLOURS.

This chapter discusses how spontaneous fermentation affected the mineral content, functional properties, thermo-pasting properties, and microstructure of finger millet flours. This chapter has already been published as an article in the ***Foods*** journal.

CHAPTER FOUR: NUTRITIONAL QUALITY, ANTIOXIDANT, MICROSTRUCTURAL AND SENSORY PROPERTIES OF SPONTANEOUSLY FERMENTED GLUTEN-FREE FINGER MILLET BISCUITS.

This chapter discusses how proximate compositions, minerals, polyphenols, antioxidant activity, physical properties, and sensory properties of gluten-free finger millet biscuits were impacted by spontaneous fermentation. This chapter has also been published as an article in the ***Foods*** journal.

CHAPTER FIVE: GENERAL CONCLUSION AND RECOMMENDATIONS

This is the dissertation's final chapter, and it includes a well-informed conclusion based on the studies conducted. It also recommends future studies focusing on finger millet value-added food products that needs to be produced for commercialisation.

CHAPTER 1: INTRODUCTION

1.1 Background

Millets are cereal grains that are commonly cultivated for human consumption and fodder in the globe (Dayakar *et al*. 2017). They provide essential nutrients, and their protein level is comparable to or better than wheat, maize, sorghum, and rice (Kumar *et al.,* 2018). Sorghum and pearl millet are two (2) major millets and other millets such as finger millet (*Eleusine coracana*), kodo millet (*Setera italic*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrense*) and barnyard millet (*Echinochloa esculenta*) are considered small millets (Chauhan *et al.,* 2018; Khare *et al.,* 2020).

Finger millet (FM) is an ancient crop having historical, cultural, and nutritional value, especially in Africa and Asia (Sood *et al.,* 2019). The grains contain high levels of protein, dietary fibres, and carbohydrates (Gull *et al.,* 2016). They are a rich source of minerals such as calcium, iron, and phosphorus (Devi *et al.,* 2014). Essential amino acids including lysine, threonine, and valine as well as health-promoting compounds such as phytochemicals are also present in the FM grains (Owheruo *et al.,* 2019; Maharajan *et al.,* 2021; Nakarani *et al.,* 2021).

The grains are staple foods in third world countries mostly in Africa and Asia (Pradhan *et al.,* 2019; Ramashia *et al.,* 2019; Anitha *et al.,* 2021). They are used to produce foods such as *chapatti* (bread), *imbali* (thin porridge), and dumplings (Almaski *et al.,* 2019; Ebere *et al.,* 2021). Malted FM flour has been utilised to prepare easy to digest foods such as infant food. Because of its low glycaemic index and gluten-free (GF) properties, FM can also be used to prepare foods for individuals who are suffering from diabetes and celiac disease, respectively.

Celiac disease is a chronic disease caused by consumption of gluten-containing foods such as wheat, barley, and rye (Stachurska *et al.,* 2019; Elsahoryi, 2021). It is classified as a long-term illness, and the sole treatment is consumption of gluten-free food (Aly and Saleem, 2015). However, there is a problem with most GF foods because they are made from refined flours that lack minerals and other essential nutrients (protein & dietary fibre). Because FM is high in micronutrients, it can be used as a potential GF material to make micronutrient-rich GF products to address nutritional challenges in GF diets (Kumar *et al.,* 2018; Maharajan *et al.,* 2021). With shortage of specialised equipment's in underdeveloped countries to process FM into value-added food products.

Fermentation can be employed in the preparation of the FM flours to boost the nutrients, potential health benefits, and mineral accessibility of GF finger millet food products such as biscuits. It has been reported that fermentation decrease antinutritional factors (ANFs) and increase nutrients bioavailability (Marsh *et al.,* 2014; Chinenye *et al.,* 2017). It has also been found to enhance the protein, dietary fibre, and minerals in pearl millet and foxtail products (Nami *et al.,* 2019; Chu *et al.,* 2019). Fermentation has been shown to alter the polyphenol content, microstructural and sensory properties of pearl millet biscuits (Adebiyi *et al.* 2016; Adebiyi *et al.,* 2017; Awolu *et al.,* 2017).

However, little information is known on how fermentation affect the physicochemical, microstructural, and sensory properties of FM gluten-free biscuits. Therefore, the attempt to utilise fermented FM flours to produce GF biscuits may be of great significance as this may help increase the knowledge about FM as well as promoting the commercialisation of GF products to benefit individuals who are suffering from celiac disease, diabetes, hypertension, and heart diseases. Hence, the aim of this study was to determine nutritional quality, macrostructural properties, and consumer acceptability of biscuits obtained from spontaneously fermented FM flours.

1.2 Statement of the research problem

Commonly available biscuits are obtained from wheat flour, which has high glycaemic index and lower phenolics, dietary fibre, minerals, and vitamins (Ho *et al.,* 2013). Wheat flour also contain gluten, which causes celiac disease. Celiac disease is a chronic disease caused by eating gluten-containing foods such as wheat, barley, and rye (Morsheli *et al.,* 2014). Finger millet grains are gluten-free and have high phenolics, dietary fibre and micronutrients. However, the grains are neglected, under researched, and undervalued (Yemets *et al.,* 2020) despites their nutritional value and potential health benefits. This could be due to a lack of information and knowledge regarding FM grains, notably their nutritional, microstructural, and baking properties, and how they are impacted by spontaneous fermentation.

1.3 Justification of the study

The accomplishment of this project may expand the utilisation of minor grains such as FM in the baking industry to produce products that may be nutritionally rich in terms of fibre contents and micronutrients. This project’s success may also create an opportunity for FM to enter in the baking industry to produce gluten-free biscuits with enhanced nutritional value for people with diabetes and celiac disease. Because FM grain/flour is gluten-free and has a low glycaemic index (GI), its utilisation in multigrain-produced food products may reduce GI which may be good for diabetic people as well those with hypertension, and heart diseases. The use of FM for biscuits production may boost the availability of value-added products in the market. Lastly, this project may help increase knowledge about FM and promote commercialising of FM food products.

1.4 Aim and specific objectives of the study

1.4.1 Aim of the study

- The aim of this study was to determine the nutritional quality, microstructural properties and consumer acceptability of biscuits obtained from spontaneously fermented FM flours.

1.4.2 Specific objectives

To investigate the impact of spontaneous fermentation on the:

- Functional and thermo-pasting properties of finger millet flours.
- Microstructural properties of finger millet flours and biscuits.
- Proximate compositions of finger millet biscuits.
- Mineral compositions (calcium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, & zinc) of finger millet flours and biscuits.
- Polyphenols and antioxidant activity of finger millet biscuits.
- Physical properties (Colour attributes, diameter, thickness, weight, spread ratio, & texture) of finger millet biscuits
- Consumer acceptability of finger millet biscuits.

1.5 Hypotheses

1.5.1 Alternative hypotheses

- Spontaneous fermentation may influence mineral compositions, functional, thermo-pasting, and microstructural properties of finger millet flours.
- Spontaneous fermentation may influence the nutritional quality, antioxidant, microstructural, and sensory properties of gluten-free finger millet biscuits.

1.5.2 Null hypotheses

- Spontaneous fermentation may not influence mineral compositions, functional, thermo-pasting, and microstructural properties of finger millet flours.
- Spontaneous fermentation may not influence the nutritional quality, antioxidant, microstructural, and sensory properties of gluten-free finger millet biscuits.

CHAPTER 2: LITERATURE REVIEW

2.1 Background of finger millet grains

Finger millet (FM) is an important cereal grain food for mostly people living in poor rural areas. It is one of the ancient grains, which is speculated to have originated in East Africa (Odeph *et al.,* 2020). The grain's (Figure 2.1) special characteristic is its ability to adapt to varied environmental conditions and have the highest productivity as compared to other millets (Pearl millet, little millet, & Kodo millet) (Gull *et al*., 2014). The cultivation of FM accounts about 12% of world millet area in African and Asian countries (Kumar *et al.,* 2016). Table 2.1 shows some few sub- Saharan African countries that produce finger millet grains. Nigeria produces the most finger millet grains, whereas South Africa produces the least.

Figure 2.1: Finger millet grains (https://florafoods.in/product/ragi-finger-millet/, Accessed, 02 September 2020).

Table 2.1: Sub-Saharan African producers of finger millet

Country	Production (tons)
Nigeria	5,000,000
Niger	2,955,000
Mali	18785271
Burkina Faso	1,109,000
Sudan	1,090,000
Ethiopia	1125958
Chad	582,000
Senegal	572,155
Zimbabwe	88000
South Africa	6340

(Sarita & Singh, 2016; Ramashia *et al.,* 2019; Ramashia *et al.*, 2021).

Tanzania, Kenya, Uganda, Rwanda, Burundi, Sudan, Madagascar, and Malawi are also among Africa's producers of FM (Sarita & Singh, 2016; Ramashia *et al.,* 2019). Asian countries that cultivate FM include India, Nepal, Bangladesh, and Sri Lanka (Gull *et al.,* 2016; Rathore *et al.,* 2019). The largest producer of FM is India (Sarita & Singh, 2016).

2.2 Finger millet grain structure

The grain (Figure 2.2) kernel shape of FM can be globular, oval, and spherical, and its size ranges from 1 to 1.8 mm (Gull *et al*., 2015). Its components include pericarp, germ, and endosperm. The pericarp is easily removable by soaking in water or by rubbing or through threshing. The seed coat of FM contains five layers and are all fused to the endosperm and According to Shobana *et al.* (2013), this could be one of the causes behind FM's greater dietary fibre content. The grain’s coat, endosperm, and germ (embryo) constitute approximately 15% of the kernel weight (Gull *et al.,* 2015).

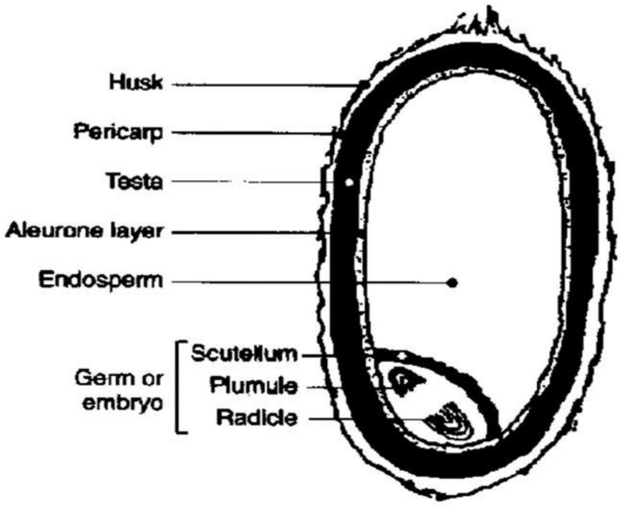

Figure 2.2: Structure of finger millet grain. Ramashia *et al*. (2019).

Endosperm is the soft and fragile part of the FM grain that is divided into three (3) areas: the peripheral area, the corneous area, and the chalky or floury section (Syeunda *et al.,* 2021). The chalky area component of endosperm makes up about 83% of the grain. The grain’s endosperm is mostly made up of starch granules (Gull *et al.,* 2016). The peripheral endosperm cell contents are so densely packed that the protein bodies leave distinct indentations in the starch granules (Obilana, 2013). According to Shobana *et al.* (2013), the corneous component of endosperm has well-organized starch granules that are inside the cell wall, whereas flour-containing endosperm has loose starch granules. The FM germ is relatively small, and it is in a depression surrounded by a distinctive ridge that runs around the entire germ. The stylar is on the kernel's opposite side from the hilum, which is next to the germ. The scutellum layer, which is the location of protein structures in the FM grain, segregates the scutellum from the endosperm, where flour is found. The FM grain's testa is attached to endosperm which makes it difficult to mill (Siwela, 2009).

2.3 Finger millet nutritional composition

The FM grain is rich in protein (6-8%), starch (65-75%), Fat (1-1.7%), minerals (2-2.25%), and dietary fibre (15-20%) (Shobana *et al.,* 2013; Gull *et al.,* 2016). It has a higher calcium, dietary fibre, and some micronutrients content than wheat, sorghum, rice, and maize (Table 2.2). Compared to other cereals such as barley, maize, wheat and rice, FM is rich in polyphenols (Chandra *et al.,* 2016). Gull *et al.* (2015) found that the FM seed coat is high in phenolic compounds, minerals, and dietary fibre.

Table 2.2: Nutritional compositions of some major cereals and millets (g/100)

Nutrients	Rice	Wheat	Maize	Sorghum	Pearl millet	Finger millet	Little millet
Protein	7.9	11.6	12.1	10.4	11.8	7.3	9.7
Fat/lipid	2.7	2.0	4.6	3.1	4.8	1.3	5.2
Ash	1.3	1.6	1.8	1.6	2.2	3.0	5.4
Crude fibre	1.0	2.0	2.3	2.0	2.3	3.6	7.6
Carbohydrates	76.0	71.0	73.0	70.7	67.0	72.6	60.9
Thiamine	0.41	0.41	0.15	0.33	0.38	0.42	0.30
Riboflavin	0.01	5.46	0.15	0.1	0.21	0.19	0.09
Niacin	1.62	5.5	1.77	3.7	2.8	1.1	3.2

(Saleh *et al.,* 2013; Prajapati *et al.,* 2019).

2.3.1 Finger millet protein and essential amino acids

Finger millet as shown in Table 2.2, has the lowest protein content when compared to other cereals. Albumins and globulins constitute about 8–15% of total proteins (Gull *et al.,* 2015). The prolamin part of albumin has a higher concentration of leucine, proline, glutamic acid, isoleucine, valine, and phenylalanine, but a lower amount of arginine, glycine, and lysine (Shaji & Sripriya, 2017). The essential amino acids are the primary determinants of protein quality. Amino acids (Table 2.3) of FM includes tryptophan (13%), threonine (3.1%), methionine (2.9%), lysine (2.5%), isoleucine and leucine (4%) (Gull *et al.,* 2016). According to Saleh *et al.* (2013), FM' s essential amino acids are balanced as compared to other millets because it has higher valine, threonine and lysine. The FM grain has a higher concentration of valine, lysine and threonine which can be utilized to boost formulated foods (Panghal *et al.,* 2019).

Table 2.3: Amino acids and minerals of finger millet

Amino acids	**g/100 g protein**	**Source**
Alanine	6.1 – 6.2	Amadou *et al.,* 2013
Arginine	2.77 – 4.5	Ramashia *et al.,* 2018
Aspartic acid	5.7	Thapliyal & Singh, 2015
Cystine	1.7 – 2.6	Sema-Saldivar, 2010
Histidine	2.2	Ramashia ,2018
Glutamic acid	20.3 – 27.1	Sema-Saldivar, 2010
Glycine	3.3	Thapliyal & Singh, 2015
Isoleucine	4.3	Thapliyal & Singh, 2015
Leucine	6.6 – 9.5	Palanisamy *et al.,* 2012
Lysine	2.2	Thapliyal & Singh, 2015
Methionine	2.5 – 3.1	Palanisamy *et al.,* 2012
Phenylalanine	4.1 – 5.2	Amadou *et al.,* 2013
Proline	7.0 – 9.9	Amadou *et al.,* 2013
Serine	5.3	Thapliyal & Singh, 2015
Threonine	3.4 – 4.2	Ramashia *et al.,* 2018
Tryptophan	1.1 – 1.5	Palanisamy *et al.,* 2012
Tyrosine	3.6	Thapliyal & Singh, 2015
Valine	4.9 – 6.6	Ramashia ,2018
Minerals		
Calcium	317- 398	Udeh *et al.*, 2017
Chlorine	84	Ramashia, 2018
Copper	0.18-0.79	Chandra *et al.*, 2016 & Ramashia *et al.*, 2018
Iron	Iron	Prajapati *et al.,* 2019
Magnesium	78- 48.43	Chandra *et al.*, 2016
Manganese	17.61- 48.43	Shimelis *et al.*, 2009 & Ramashia *et al.*, 2018
Phosphorus	150- 250	Chandra *et al.*, 2016
Potassium	0.43-0.49	Chandra *et al.*, 2016
Sodium	49	Siwela, 2009 & Ramashia *et al.*, 2018
Zinc	2.3	Prajapati *et al.,* 2019

2.3.2 Lipids

The lipid content of FM is about 2.7% (Table 2.2). The major constituents of FM lipids include linoleic, palmitic, and oleic (Gull *et al.,* 2016). It has about 72% of total lipids and most of them are triglycerides, and about 12% as glycolipids, and 6% as phospholipids (Gull *et al*., 2016). Saturated and unsaturated fatty acids make up 25.6 and 74.4 % of the total fatty acids, respectively (Thapliyal *et al.,* 2015). The triglycerides of lipids of FM are said to minimize of the risk of duodenal ulcers (Gull *et al.,* 2016).

2.3.3 Carbohydrates

The FM grain is rich in carbohydrates (Table 2.2), and it constitutes non-starchy polysaccharides, free sugars, and starch. The grain's total carbohydrate content is in the range of 72 to 79.5% (Thapliyal *et al.,* 2015). Starch is the primary component of carbohydrates (Hamaker *et al.,* 2019). Shobana *et al.* (2013) reported 1.4–1.8% cellulose, 59.5–61.2%, starch, 6.2–7.2% pentosans, and 0.04–0.6% lignins. Amylopectin accounts for around 80 to 85 percent of FM starch, with amylose accounting for the remaining 15 to 20 percent (Singh & Raghuvanshi, 2012). The total carbohydrates in FM are made of about 30% of non-starch polysaccharide (Ambre *et al.,* 2020).

2.3.4 Dietary fibres

Finger millet as shown in Table 2.2, has high proportion of dietary fibre than most of other cereals (Kaur *et al.,* 2020; Ambre *et al.,* 2020). Dietary fibres are classified as either water soluble or water insoluble (Gidley & Yakubov, 2019). Devi *et al.* (2014) reported the total dietary fibre content of FM grain to be 19.10%. Gull *et al*. (2015) reported that FM grain contains 1.4% of soluble fibre and 15.7% of insoluble fibre. About 22% dietary fibre, 2.5% soluble dietary fibre and 19.7% insoluble dietary fibre of FM were reported by (Shobana & Malleshi, 2007).

2.3.5 Vitamins

Vitamins as shown in Table 2.2, are classified as fat soluble or water-soluble vitamins, and shortage of either leads in vitamin deficiencies, which cause heath concerns (Ramashia, 2018; Fatima *et al.,* 2019). The lipo-soluble vitamins and water-soluble B-vitamins are largely contained in the aleurone and germ, respectively (Garg *et al.,* 2021). Vitamin B complexes that are found in FM grain include B1, B2, B3, B6 and B9. These vitamins are important because they maintain the health of human body (Ramashia *et al.,* 2018). The deficiencies caused by lack of vitamins include pellagra and beriberi. Akhtar *et al*. 2011 reported that, in developing countries, the deficiency of vitamin B3 is a serious a serious problem.

2.3.6 Mineral contents

Finger millet has a higher calcium content as compared to other millets (Maharajan *et al.,* 2021). Udeh *et.al.* (2017) reported that calcium content of FM ranges from 317.1 to 398.0 mg/100g. Wafula *et al*. (2018) reported the calcium content of FM as 330 mg/100g. Table 2.3 shows the mineral composition of finger millet. Calcium helps in strengthening the bones (Hassan *et al.,* 2021). In comparison to many millets, FM grains contain higher levels of phosphorus (P) and magnesium (Karuppasamy, 2015). The absorption and utilisation of minerals in the body prevent cancer, diabetes, high blood pressure, cardiovascular diseases, and mineral deficiencies (Kaur *et al*., 2014; Ramashia *et al*., 2018). The deficiency of iron and calcium lead to bone disorder and anaemia, respectively (Anitha *et al.,* 2021; Jagathi *et al.,* 2021). These deficiencies can be eradicated by the introduction of FM in people's diet.

2.3.7 Antioxidant properties of finger millet

Antioxidant compounds are significant because they stabilize lipid and suppresses oxidative stress, which is linked to cancer and aging (Devi *et al*. 2014). The examples of antioxidant compounds include phenolic acids and their derivatives, tannins and flavonoids as indicated in Table 2.4. These antioxidants are all present in the seed coat of FM and they act as free radical terminators, singlet oxygen quenchers, and metal chelators (Devi *et al.* 2014).

Table 2.4: Polyphenols of finger millet cultivars

Finger millet varieties	TPC (mg GAE/100g)	TFC (mg CE/100g)
Brown finger millet	302.42	197
Red finger millet	292.29	202.94
White finger millet	136.4	115.8

(Xiang *et al.* 2019; Ofosu *et al.* 2020). TPC= Total phenolic content; TFC= Total flavonoid content; GAE= Gallic acid equivalents; CE= Catechin equivalents.

Phenolic compounds can be defined as any compounds that contain the benzene ring that has hydroxyl groups (Lin *et al.,* 2016). Phenolic compounds such as benzoic acid (Figure 2.3), are one of the most diverse classes of phytochemicals found in plant diets and hence play an essential role in human nutrition (Chandrasekara &Shahidi 2012; Udeh *et al*., 2017). The genotypes of FM differ in terms of total phenolic content (Devi *et al*., 2014; Karki & Kharel, 2012; Xiang *et al.,* 2019). They are two types of phenolic compounds which include soluble phenolics and insoluble phenolics (Shahidi & Yeo, 2016). The soluble phenolics are those that are extractable in aqueous solution. Insoluble phenolics, also known as the bound phenolic compounds, are left over residue after extraction of soluble phenolic compounds that are esterified to cell walls and can only be extracted using acid or base hydrolysis (Anokwuru *et* *al,* 2018). Hydroxybenzoic acid derivatives, hydroxycinnamic acid derivatives, and flavonoids with a basic skeleton of C_6–C_1, C_6–C_3, and C_6–C_3–C_6 are the three types of phenolics present in FM (Gull *et al.,* 2014). The FM grain is recognised as nutri-cereal because of its plentiful number of phytochemicals (Singh & Raghuvanshi, 2012). Procatechuic, gallic, caffeic, ferullic, and quercetin are phenolic chemicals that have been found in FM (Chethan & Malleshi, 2007). White FM typically has lesser phenolic chemicals, whereas brown FM has substantially higher values (Siwela *et al*., 2007). The brown FM cultivar contains a higher amount of polyphenols than the white cultivar (Chethan & Malleshi, 2007).

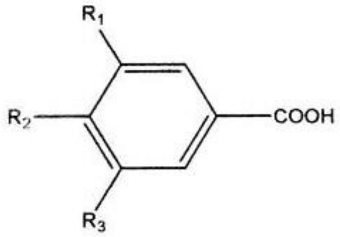

Figure 2.3: Structure of phenolic acid: Benzoic acid (Liu, 2004; Udeh *et al.,* 2017).

Flavonoids as shown in Figure 2.4, are powerful antioxidants that aid in the prevention of chronic diseases (Okarter *et al*., 2010; Xiang *et al.,* 2019). They are a compound of C6-C3-C6 skeleton that consist of an aromatic ring bound together by a three-carbon link (Santos-Buelga & faleciano, 2017). In cereals, the location of flavonoids is pericarp (Liu *et al.,* 2013). The examples of flavonoids include flavanols, anthocyanins, flavones, and flavanones. The most researched flavonoids in grains are anthocyanins, which are water-soluble (Francavilla & Joye, 2020). The flavonoids of FM are also mostly soluble and the highest when compared to other millets (Okwudili *et al.,* 2017). Flavonoids, except for anthocyanins, and flavonols are either yellow or brown (Shoji, 2007). Flavonoids in FM coat have multiple functions such quenching singlet oxygen and eliminating harmful metals in the body (Gull *et al.,* 2014). Varietal variation exists in flavonoids of FM. According to Ofosu *et al.* (2020) total flavonoid content of FM types varied from 101.3 to 115.8 mg CE/100g. Total flavonoids concentration in different FM cultivars was observed to range from 90.24 to 202.94 mg CE/100g by Xiang *et al*. (2019).

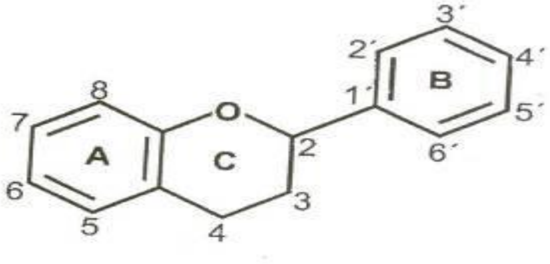

Figure 2.4: General structure of flavonoid (Siwela *et al.,* 2007).

2.4. Effects of food processing technologies on microstructural properties of food using scanning microscopy electron

The microstructure of food can be defined as the spatial arrangement of the cell and the intercellular space in food materials (Karim *et al.,* 2018). The micro-structured foods include meat, vegetables, fruits, and bakery products. During food processing there is destruction of the existing food structures and formation of new structures. The structural changes that take place during food processing can lead to the degradation of food quality. Rahman *et al.* (2016) also reported that process conditions impact the quality and stability of food products as indicated in Table 2.5. Therefore, it is of great importance that during food processing, food microstructures are preserved.

Table 2.5: Effects of processing on the microstructural properties of food products using scanning microscopy electron.

Food	Food quality and microstructure as affected by food processing.	References
Muffins	Gelatinised starch granules of whole wheat flour baked muffins were embedded in protein matrix while the starch granules of pearl millet flour muffin formed a film like structure.	Umashankar *et al.,* 2016
Biscuits	Fermentation and malting caused starch granules to have spherical and polygonal shapes. About 40% native FM flour biscuits starch were seen embedded in protein and starch matrix, while 40% germinated FM were trapped in gluten matrix	Adebiyi *et al.,* 2016; Shimray *et al*., 2012
Finger millet	Hydrothermal treatment changed the starch granules pattern into coherent mass, while the high temperature treated starch formed honeylike comb.	Dharmaraj *et al.,* 2014
	The shape of starch granules of FM and pearl millet flour looked disrupted because of the structural changes they had undergone during milling.	Gull *et al*., 2016
Pearl millet	Fermentation altered the size and shape of starch granules.	Adebiyi *et al.,* 2016
Pasta	Starch granules in cooked pasta were coated in protein matrix, whereas the starch granules in uncooked pasta exhibited a smooth surface. Starch granules were embedded in gluten network	Gull *et al*., 2016; Rajeshwari *et al.,* 2013

2.5 Finger millet grain/flour utilisation and potential health benefits.

Finger millet flour is an ideal substitute for wheat, rice, and other grains. It can be used to make multigrain flour, which is also known as composite flour. The grain can also be used to make traditional beer and porridge (Ramashia *et al.,* 2019). In other countries, the grains are used to make products such as *dosa* (Indian fermented pan cakes)*, roti* (flat bread) and *idli* (steamed cake) after being processed by traditional methods including fermentation, malting, and grinding (Shobana *et al*., 2013). The grain is also beneficial to patients who suffer from diabetes and heart problems (Jagati *et al.,* 2021).

Shuckla & Srivastava (2014) studied glycaemic index of FM incorporated diet and reported that it lowers the glucose levels upon consumption, owing it to the high fibre content of FM. The inclusion of ANFs in whole FM flour lower starch digestion and absorption, could explain why whole FM-based diets have a low GI response (Gull *et al*., 2015). The grain also contains phytochemicals such as phenolic and flavonoid compounds (Jamra *et al.,* 2021). These compounds aid in the prevention of cancer and cardiovascular disease, as well as minimizing the detrimental effects of aging (Siwela *et al.,* 2010, Kumar *et al.,* 2021).

The consumption of FM delay nutrient absorption, lower blood lipids, increases faecal bulk, and prevent colon cancer (Karki *et al*., 2020). The slow digestion of FM helps one keep away from the excessive intake of calories. The FM grain has tryptophan which suppresses appetite and aids in weight management (Bhasker *et al.,* 2017). Coulibaly *et al.* (2011) stated that FM phytochemicals including phytic acid and phytate are linked to lowering cholesterol and a lower cancer risk, respectively. The consumption of FM, due to its high fibre and polyphenol content reduces the risk of gastrointestinal tract disorders, and diabetes mellitus.

These potential health benefits can be ascribed in part to phytochemicals, a class of potentially cancer-preventive substances that includes antioxidants found in high concentrations in foods like millets (Izadi *et al*., 2012). Calcium, which is abundant in FM, is important for pregnant women, growing children, and the elderly (Anitha *et al.,* 2021), as well as persons with malnutrition, obesity, and diabetes (Ramashia, 2018). It also helps in strengthening bones (Ambati *et al.,* 2019).

Phosphorus, magnesium, and iron are among of the other minerals found in FM, and they all serve significant roles in the human body (Ramashia, 2018). Ambati & Sucharitha (2019), reported that phosphorous is involved in the growth of bodily tissue as well as energy metabolism. The FM grain is also high in amino acids which are vital for the body's day-to-day functioning and tissue repair (Rathore *et al.,* 2019; Mitharwal *et al.,* 2021).

2.6 Technological processes of finger millet.

Prior to consumption, FM like all other cereal grains, undergoes food processing. Processing technologies including milling, soaking, malting, fermentation, cooking, roasting, and decortication are used to enhance the functional, nutritional, and sensory qualities, as well as the convenience, of cereal grains (Rani *et al.,* 2018; Saleh *et al.,* 2019). However, this study was focusing on the fermentation, and milling of FM as well as baking of biscuits from FM flours.

2.6.1 Fermentation

Spontaneous fermentation is a metabolic process that uses microorganisms to break down complicated materials into simpler forms (Rathore *et al.,* 2019). It is an old processing method used to make foods and beverages (Zhao *et al.,* 2019) and has also been reported to be useful in the preparation and processing of millet into several other edible forms, causing desirable changes in millet's components, composition as well as the structure (Chinenye *et al.,* 2017). Fermentation does not only improve the taste, but it also increases the nutritional value of food by reducing ANFs (Verma & Patel, 2013). The millets protein quality is low in terms of lysine and tryptophan content (Anitha *et al.,* 2020).

It has been found to enhance the protein quality by increasing lysine and tryptophan in cereal grains (Rathore *et al.,* 2020; Gwer *et al.,* 2020; Tatiana *et al.,* 2021). Spontaneous fermentation of FM using microflora has been reported to increase the bioavailability of iron, zinc phosphorus, and calcium by reducing tannins, trypsin inhibitor and tannins (Jaybhaye *et al.,* 2014). Saleh *et al*. (2018) reported the enhancement of crude protein and reduction of crude fat and fibre in *rabadi* due to fermentation. Commercial industries use fermentation to produce value-added products. The process of fermentation is also economic because it helps preserve food products. Traditional fermented foods include baked and fried pancakes, thin porridge (*ambali*) and thick porridge (dumpling) (Ramashia, 2018).

2.6.1.1 Microbiology and biochemistry of lactic acid fermentation of cereals

Lactic acid bacteria are a beneficial group of microorganisms that are involved in the preservation of food by lactic acid fermentation (Petrova & Pretrov, 2020). These bacteria are Gram-positive, non-sporulating, non-pigmented and non-motile rods and cocci, most of which are non-respiring but aerotolerant anaerobes (Liptáková *et al.,* 2017). Based on the end product of fermentation, Lactobacillus species are categorized into three groups: obligate homofermenters, facultative heterofermenters, and obligate heterofermenters. Obligate homofermenters can only ferment hexoses to lactate while facultative heterofermenters convert pentoses through the phosphoketolase pathway and hexoses through the Embden ‐ Meyerhof ‐ Parnas glycolytic pathway into lactate, acetate, formic acid, and ethanol (Salminen & Von Wright, 2004). In the case of obligatory heterofermenters, the primary products of fermentation include lactic and acetic acid (or ethanol), carbon dioxide, and hexoses and pentoses (Liptáková *et al.,* 2017). *Lactobacillus* and *Pediococcus* sp are used as fermenting microorganism in the production of Indian fermented FM food product as shown in table 2.6 (*Koozhu*) (Satish Kumar *et al*., 2010).

Table 2.6: Some of the examples of foods obtained by lactic acid fermentation of cereals

Cereal	**Product**	**Used LAB species**	**Reference**
Finger millet	*Koozhu*	*Lactobacillus* & *Pediococcus* sp	Satish Kumar *et al*., 2010.
Sorghum	*Ogi*	*L. plantarum*	Agativ *et al.,* 1998.
Maize	Mahewu	L. plantarum & *L. fermentum*	Pswarayi, & Gänzle, 2019.
Rice	Noodle	*Lactobacillus* & *Streptococcus*	Pisitkul & Rengpipat, 2014.
Wheat	Bread	*L. fermentum*	Corsetti *et al.,* 2004

2.6.2 Milling

Finger millet is commonly ground into flour for use in food preparations. It is cleaned to get rid of foreign matters such as chaffs, stalks, and stones before the milling process begins (Ambre *et al.,* 2020). Milling is done primarily to separate three (3) components (Bran, germ, & endosperm) of the grain from each other (Rani *et al.,* 2018). It can be done in one of two ways: (1) turning the whole grain to flour without removing any component of the grain or (2) employing differential milling to separate the grain into various portions (Oghbaei *et al.,* 2016). For instance, FM could be milled as whole FM flour or subjected to roller milling to produce refined FM flour, bran, and germ. Milling breaks down cell walls, increasing the amount of antioxidant compound available in the flour. It can also result in a large loss of nutrients, vitamins, and minerals, because these substances are mostly found in the bran portion of the (Yousaf *et al.,* 2021).

2.7 The role of ingredients in biscuits production

Biscuits are often made with wheat flour, oil, and sugar as the primary ingredients. Each ingredient has a specific role to play in biscuit production. Sugar imparts sweetness to baked goods, as well as colour and texture (Mamat & Hill, 2018). Fats and oil also essential in the production of biscuit as they act as lubricants and influence the machinability of dough during processing, and the gustatory and textural qualities of baked biscuit (Mamet and Hill, 2018). The leavening agent used in biscuit production such as sodium bicarbonate makes biscuit crispy and porous. Water is an essential ingredient of biscuit as it helps dissolve chemicals, salt, flavour and distribute them throughout the dough (Misra & Tiwari, 2014). Minor ingredient such as flavour (Vanilla and chocolate flavour) is added in the biscuit production to impart flavour to the final product (Misra & Tiwari, 2014).

2.8 Baking

Baking is a type of traditional cereal-based cooking that plays a significant role in today's food processing industry (Devi *et al.,* 2021). It involves the use of a hot air oven to transmit heat by conduction, convection, and radiation (Chhanwal *et al.,* 2019). Physical and biological changes such as volume expansion, steam evaporation, crust formation, colour development, and structure construction are all caused by baking (Jerome *et al.,* 2019). The Maillard process and caramelization are non-enzymatic browning mechanisms that contribute to the formation of colour and aroma during baking (Consoli *et al.,* 2018). The Maillard reaction occurs when proteins, reducing sugars, amino acids, and compounds that contain nitrogen are heated (Shendurse & Khedkar, 2016).

Reducing sugars and amino acids trigger the Maillard process, which releases a variety of aromatic taste chemicals and higher molecular weight brown melanoids. Caramelization, on the other hand, is a term used to describe a complicated set of reactions that occur when carbohydrates, particularly sucrose and reducing sugars, are heated directly (Ghnimi *et al.* 2021). The colour of a biscuit is only determined by temperature, hence a first-order kinetic model based on average moisture content and temperature was created to predict cracker surface lightness variation throughout baking (Odeh *et al.,* 2018). Consumer acceptability of bakery products such as biscuits is influenced by their surface colour (Millar *et al.,* 2017).

2.9 Biscuits

Biscuits are a popular ready to eat baked product that are consumed around the world (Adeola & Ohizua, 2018). The flour, shortening, leavening, and milk or water are used to make them. The amount of ingredients used in the production of biscuits impacts the quality of final baked biscuits (Arepally *et al.,* 2020). Biscuits are consumed as snack or with drinks by all age groups and have long shelf life. Because biscuits have a long shelf life, they may be produced and distributed on a big scale (Hassan *et al.,* 2013). They are typically made with wheat flour, but they can also be made with various cereal grain flours such as finger millet and maize flours among others (Sambavi *et al.,* 2015; Di Cairano *et al.,* 2018).

2.10 Texture profile of finger millet biscuits

Texture profile is the measurement and description of the textural properties of food. Texture is one of the sensory qualities that is considered crucial in new food product development (Adebiyi *et al*., 2016). Texture can be measured by a trained sensory panel or using specialized equipment. It was designed as a two-cycle compression performed to simulate successive “chews” (Bourne,2002). Parameters that are analysed in the texture profile of food include chewiness, hardness, cohesiveness, and springiness (Morey and Owens, 2017). Texture profile analysis tests entails submitting the sample to many compression forces and recording the resulting curve of force and distance (Megahey *et al.,* 2005). For most cereal baked items, a probe of specific diameter is used to evaluate the hardness (Rosenthal, 1999) and the peak force is recorded as the hardness of that baked item. Adebiyi *et al*. (2016) analysed biscuits made from 100% pearl millet flour and found that fermentation increased the toughness of the biscuits.

2.11 Conclusion

In this work, an overview of FM and fermentation was thoroughly examined. It is possible to conclude that FM is a good source of essential nutrients such as proteins, dietary fibre, amino acids, vitamins, minerals, and health-promoting compounds such as phytochemicals, which are known to prevent cancer, heart disease, and cardiovascular disease. The grain nutritional value can be increased further by using processing technologies such as fermentation, which has been reported to bring desirable changes in cereal grains. The grain is also gluten-free, which is advantageous for celiac patients. Despite all the nutritional benefits of the grain, it is still underutilized, unresearched, and there are limited food products if not none, that are made from it on the market. As a result, this grain must be carefully explored, particularly its baking and cooking properties, so that there are more gluten-free FM value-added food products on the market, which may assist local farmers, strengthen Africa's economy, and aid in the accomplishment of food security in Africa.

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CHAPTER 3: MINERAL CONTENT, FUNCTIONAL, THERMO-PASTING AND MICROSTRUCTURAL PROPERTIES OF SPONTANEOUSLY FERMENTED FINGER MILLET FLOURS

Abstract

Finger millet is a cereal grain which is superior to wheat and rice in terms of dietary fibre, minerals, and micronutrients. Fermentation is one of the oldest methods of food processing, and it has been used to ferment cereal grains such as finger millet (FM) for centuries. The aim of this study was to investigate the impact of spontaneous fermentation (SF) on mineral content, functional, thermo-pasting, and microstructural properties of light- and dark-brown FM flours. Spontaneous fermentation exhibited a significant increase in the macro-minerals and micro-minerals of FM flours. In terms of functional properties, SF decreased the packed bulk density and swelling capacity, and it increased the water/oil absorption capacity of both FM flours. Spontaneous fermentation had no effect on the cold paste viscosity of FM flours. However, significant decreases from 421.61 to 265.33 cP and 320.67 to 253.67 cP were observed in the cooked paste viscosity of light- and dark-brown FM flours, respectively. Moreover, SF induced alterations in the thermal properties of FM flours as increments in gelatinisation temperatures and gelatinisation enthalpy were observed. The results of pasting properties exhibited the low peak viscosities (1709.67 and 2695.67 cP), through viscosities (1349.67 and 2480.33 cP), and final viscosities (1616.33 and 2754.67 cP), along with high breakdown viscosities (360.00 and 215.33 cP) and setback viscosity (349.33 and 274.33 cP), of spontaneously fermented FM flours. Scanning electron microscopy showed that SF influenced changes in the microstructural properties of FM flours. The changes induced by SF in FM flours suggest that flours can be used in the food industry to produce weaning foods, jelly foods, and gluten-free products that are rich in minerals.

Keywords: millets; techno-functional properties; gluten-free; natural fermentation; microstructure

3.1 Introduction

Finger millet (*Eleusine coracana*) is a nutrient-dense cereal grain that is underutilised and is commonly viewed as food for poor people [1]. Globally, finger millet (FM) is important, ranking fourth after sorghum, pearl millet, and foxtail millet [2]. It is commonly cultivated in subtropical and tropical areas where it is difficult to grow other cereal crops such as maize and wheat [3]. In Africa, FM is widely cultivated in Kenya, Uganda, Ethiopia, Burundi, Malawi, Tanzania, and Zambia [4]. There are different cultivars of FM such as white, brown, and reddish cultivars [5]. The brown cultivar is used to produce traditional beer and porridge, while the white cultivar is utilised in the baking industry [6].

The grain contains no gluten and can be a good alternative for celiac patients [7]. It is high in micronutrients such as vitamins and minerals, as well as essential amino acids (tryptophan, methionine, histidine, and lysine) [8]. The layers of bran of FM consist of phenolic compounds which provide various nutritional and functional benefits [9]. Finger millet has nutraceutical characteristics, and it also has antimicrobial, antidiarrhea, antidiabetic, antioxidant, antitumour, and anti-inflammatory properties [10]. The nutritional content of FM is dependent on the processing methods and the presence or absence of antinutritional factors, including the interaction of nutrients with other food components. Antinutritional factors including oxalates, tannins, and phytic acid present in cereal grains such as FM have been reported to limit the nutrients' bioavailability [11–13]. Cereal grains are subjected to different food processing techniques in the preparation or processing stages to reduce antinutritional factors.

Fermentation, malting, decortication, soaking, and germination are just a few of the old traditional food processing technologies that decrease antinutritional factors in cereal grains [14–16]. Spontaneous fermentation (SF) is a metabolic process that uses microorganisms to break down complex materials into simpler forms [17]. It is an ancient food processing technique that has been used for a long time, which enhances the functional, thermal, and pasting properties, as well as nutritional quality of foods [18–20]. Moreover, SF conspicuously improves the nutritional composition of cereal and millet grains since it enhances protein content, digestibility, and lysine content [21].

During the SF process, flour goes through various chemical changes such as modification of sugar, softening, and starch hydrolysis. This results in an improvement of the nutritional composition of FM grain, low levels of antinutritional factors, and the enhancement of micronutrients' bioavailability [22]. Additionally, Azeez et al. [8] and Mutshinyani et al. [23] reported an increase in titratable acidity, total soluble solids, polyphenols (total phenolic and flavonoid contents), and antioxidant activity (DPPH and FRAP), along with a decrease in the pH value, of spontaneously fermented brown FM flours. The influence of SF on phenolic compounds depends on the types of grain, microorganism species, and fermentation conditions such as temperature, pH, and time. Low pH during SF extends the shelf-life of flours since the growth of most bacteria is inhibited at pH level less than 4 [9]. The decrease in pH of spontaneously fermented flours is due to soluble organic acids released from flours, which are products of lactic acid fermentation [23]. This shows that SF does impart desirable changes in the biochemical properties of food.

The functional properties of foods are important chemical and physical properties that show how structures, compositions, molecular conformation, and physicochemical properties interact with the environment, as well as the states in which they are determined and interrelated [24]. The behaviours of ingredients used for food preparation or cooking, as well as how they impact the taste and appearance of the product, are described by functional properties [25]. The functional properties of flour which include bulk density, oil, and water absorption capacity are essential determinants of bakery products' quality [26]. They affect the sensory and textural properties, as well as the shelf-life, of food products [27]. Pasting properties determine the suitability of flours for baking applications [28].

There are limited studies on how SF affects the functional and thermo-pasting properties of FM flours. Modifications in the functional and microstructural properties of pearl millet flour due to SF have been reported [29–31]. Amadou et al. [18] reported changes in the pasting and thermal properties of foxtail millet caused by SF. It is, thus, critical to try to understand the impact of SF on the different components of both varieties of FM grains, which may contribute to the body of knowledge on the grain's functionality. Such information may increase the utilisation of FM in the food industry and aid in the attainment of food security in third-world countries. Despite its superiority, FM is still underutilised in processed food products due to low usage in ready-to-eat or use food. Hence, the main aim of this paper was to investigate the influence of SF on the mineral content, as well as the functional, thermo-pasting, and microstructural properties, of FM flours.

3.2 Materials and methods

3.2.1 Materials and Reagents

About 10 kg of each of light-and dark-brown FM grains were purchased from different street vendors at the local market of Thohoyandou, Limpopo Province, South Africa. Analytical-grade chemicals used for analysis were obtained from Merk Chemicals (PTY) Ltd., Germiston, South Africa.

3.2.2 Finger Millet Flour Production

All foreign matters (soil and stones) on FM grains were washed away with tap water, and the wet grains were placed on a tray wrapped with foil, transferred to an air oven drier, and allowed to dry for 24 h at the temperature of 40 ˚C. Some of the dry grains were reduced to flour with a miller (ZM 200 Miller, Retsch, Düsseldorf, Germany) and sifted with a 500 µm mesh sieve to make native flour, which was used as the control. The control sample was placed in an airtight plastic and kept in the refrigerator at 4 ˚C until it was needed for analysis [32]. For SF, the dried grains (200 g) were dispensed in a container filled with 800 mL of distilled water and spontaneously fermented in an incubator for different times (24, 48, and 72 h), at the temperature of 28 ˚C. The water was thrown away after each fermentation phase before the grains were transferred into an air oven drier for 24 h drying (40 ˚C). Dried grains were then pulverised with a miller (ZM 200 Miller, Retsch, Düsseldorf, Germany) and sifted through 500 µm mesh sieve to produce fermented flours. The fermented flours were transferred into a polyethylene and kept at 4 °C in refrigerator for further analysis [33]. For validation of the results, flour samples were replicated three times.

3.2.3 Mineral Compositions of Fermented Finger Millet Flours

ICP emission spectroscopy (ICP-AES, Jarrel-Ash) was employed to quantify the macroelements and trace elements of FM flours as per method used by Ramashia et al. [34]. Approximately 2 g of flour samples were burned by a muffle furnace at 550 °C for 3 h until they were reduced to ashes. Thereafter, the ashes obtained were combined with 5 mL of nitric acid and 10 mL of hydrochloric acid solution. The combination was placed in the water bath for 1 h, mixed with 10 mL of HCL, and poured into a 100 mL of volumetric flask. After that, there was an addition of distilled water until a volume of 100 mL in the volumetric flask was reached. The sample was placed in ICP-AES for mineral analysis. Minerals were measured in milligrams per 100 g.

3.2.4 Functional Properties of Spontaneously Fermented Finger Millet Flours

3.2.4.1 Loose/Packed Bulk Density

The loose/packed bulk density (LBD/PBD) of FM flours was measured as per the method proposed by Amandikwa et al. [35]. About 10 g of FM flours were weighed in a weighing boat and transferred to a 25 mL measuring cylinder to obtain LBD. The PBD was obtained by severally tapping the bottom of the cylinder until a constant volume of FM flours was observed. The flour weight per flour volume was used to calculate loose BD and packed BD (g/cm^3).

3.2.4.2 Water absorption capacity and oil absorption

The water absorption capacity (WAC) and the oil absorption capacity (OAC) of FM flours were measured as per the method described by Mudau et al. [36]. About 1 g of flour sample was weighed in a weighing boat, transferred into a weighed centrifuge tube, and combined with 10 mL of water/sunflower oil. The combination was vortexed (Model 36110740, Separation Scientific, Midrad, South Africa) for 30 min at room temperature (±25 °C) and centrifuged (Rotina 380 R- Labotech Ecotherm, Midrand, South Africa) at 3000× *g*, rpm for 25 min. After centrifugation, the liquid above the sediment was poured into a beaker, and the WAC and OAC were calculated by subtracting the weight of the sample before and after the addition of water/sunflower oil from the weight of the sample. The obtained results were expressed in grams of water/sunflower oil per gram of FM flour.

3.2.4.3 Swelling Capacity

The swelling capacity (SC) of FM flours was evaluated as described by Adebiyi et al. [29]. The flour was transferred into a 100 mL graduated cylinder until the 10 mL mark in the cylinder was reached. After that, distilled water was added until the 50 mL mark of total volume was reached. The tops of the graduated cylinders were then covered tightly and inverted so that the contents could be thoroughly mixed. After 2 min, the suspension was inverted again and allowed to settle for 30 min. After 30 min, the sample's volume was taken.

3.2.5 Viscosity of finger millet flours determination

The viscosity of FM flours was examined using a Brookfield viscometer (Model RV, Brookfield Engineering, Inc.,Middleboro, MA, USA) according to a method described by Ramashia et al. [6]. About 10 g of FM flours were hydrated with distilled water (90 mL) in a beaker for 30 min. The mixture was agitated occasionally until it became a slurry of which the viscosity was measured, and the cold paste's viscosity was recorded. The viscosity of cooked paste was determined by heating the slurry until it boiled at 95 °C in a water bath. The cooked paste was cooled to 30 °C and measured, and the viscosity was recorded.

3.2.6 Thermal properties of finger millet flours determination

The thermal properties of fermented FM flours were measured through differential scanning calorimetry (DSC) (DSC 4000, Perkin-Elmer, Shelton, CT, USA). A DSC pan was placed on weighing balance, and 25 mg of FM flours were transferred into it. The pan was sealed and heated from 20 °C to 130 °C. The heating rate was 10 °C per minute. In all DSC runs, there was an empty sealed pan serving as control. The gelatinisation temperatures (onset, peak, and conclusion temperature), gelatinisation temperature range, and the gelatinisation enthalpy of FM flours were evaluated and recorded through Pyris thermal system software connected to DSC [37].

3.2.7 Pasting properties of finger millet flours determination

A rapid Visco-analyser (RVA-4, Narrabeen, Australia) was employed to analyse the pasting properties parameters of FM flours as per the method explained by Siwatch et al. [38]. Briefly, 2.5 g of FM flour was dipped in 25 mL of distilled water inside the sample canister, which was then loaded into the RVA. The contents were heated to 50 °C for 1 min and heated to 90 °C again for 1 min before cooling for 2 min at 50 °C. The heating and cooling rates were constant at 12 °C per min. All pasting viscosities or parameters were recorded by a thermocline version 3.

3.2.8 Colour Profile of Spontaneously Fermented Finger Millet Flours

Colour of FM flours was analysed using Hunter Lab colorimeter (MiniScan XE Plus, Model CM-3500d, Hunter Associate laboratory, Reston, VA, USA). The equipment was calibrated with black and white tiles. Colour reading was expressed by Hunter values for L* (lightness), a* (redness) and b* (yellowness). The following formulas were used to calculate Chroma (C), hue angle (H°), and colour change (ΔE).

$$Chroma = \sqrt{(a^*)^2 + (b^*)^2}$$

$$Hue\ (H°) = \tan^{-1}\left\{\frac{b^*}{a^*}\right\}$$

$$\Delta E = \sqrt{(L - Lc)^2 + (a - ac)^2 + (b - bc)^2}$$

where Lc = lightness of control sample, ac = redness of control sample, bc = yellowness of control sample.

3.2.9 Fourier-Transform Infrared Spectra of Fermented Finger Millet Flours

A FTIR spectrometer Nicolet 8700 (Thermo Scientific, Inc, Massachusetts, USA) was used to analyse FM flours, with wavelengths ranging from 400 to 4,000 cm^{-1}. About 0.5 g of flours was mounted on the instrument, analysed and the sample's spectra were obtained. The equipment was running 32 scans for each spectrum collected [30].

3.2.10 Microstructural Analysis of Fermented Finger Millet Flours Using Scanning Electron Microscopy (SEM)

A gold palladium layer in a coater was used to coat FM flour samples that were placed on a sample holder. A scanning electron microscopy (Model, JSM 6610-LV, Chicago, USA) was used to analyse microstructure of FM flours at 1000× magnification and 20 µm scale following a slightly modified method used by Gull et al. [40].

3.2.11 Statistical Analysis

The statistical tool SPSS 26 for windows (SPSS Inc., Chicago, Illinois) was employed to assess the experimental data. The experimental data gathered from each of the FM flour samples was measured in triplicate. The disparities between the values of means were compared through Duncan's Multiple Range Test ($p \leq 0.05$).

3.3 Results and discussion

3.3.1 Mineral Compositions of Fermented Finger Millet Flours

The impact of SF on the mineral composition of FM flours is shown in Table 3.1. The macro-minerals and some micro-minerals in the two flours increased as SF time increased. When compared to the native flours, the macro-minerals in the two flours were considerably higher at 72 h of fermentation. In light brown FM flours, the sodium (Na) content significantly increased by 86.24% and followed by magnesium (Mg), phosphorus (P), potassium (K) and calcium (Ca), which increased by 14.26%, 7.05%, 6.70% and 5.33%, respectively. The Ca, P, K, Mg, and Na values were also higher in 72 h dark brown FM flours as compared to the native flours, with increases of 7,42%, 10.97%, 11,25%, 18.44%, and 161.87%, respectively. These results are consistent with those of Azeez et al. [8], who observed an increase of Ca and K in germinated and solid-state fermented FM flours.

For micro-minerals, there was a significant decrease of manganese (Mn) by 4.11% in 72 h light brown FM flour in comparison with the native flour. The increase by 83.33%, 82.40% and 76.86% was observed in iron (Fe), copper (Cu), and zinc (Zn) of light brown FM flours. At 72 h fermentation period, the Zn and Mn content in dark brown FM flours were 12.10% and 19.43% less than the native dark brown FM flour, respectively. However, Cu and Fe were 165.90% and 57.40% significantly higher than the native flours. The increase in macro-mineral and some micro-mineral contents of fermented flours could be attributed to the decrease of antinutritional factors such as phytic acids that might have occurred during SF [8].

Mineral bioavailability can be reduced by antinutritional factors such as phytic acids, which form insoluble compounds with minerals. So, during SF, the enzymatic activities break down insoluble complexes between phytic acids and minerals resulting in an increased mineral content [24]. The improved mineral content due to SF have previously been reported [41, 42, 8]. So, the enhanced mineral contents of light brown and dark brown FM flours by SF imply that both flours may be employed in the food industry to produce food products that can address mineral deficiencies in children especially in most developing countries such as South Africa.

Table 3.1: Influence of spontaneous fermentation on macro and micro-minerals of finger millet flours.

LBFMF	**Fermentation period (h)**			
Macro minerals	**0**	**24**	**48**	**72**
Ca	373.68 ± 1.67^{a}	382.22 ± 1.34^{b}	389.57 ± 1.95^{c}	394.24 ± 1.48^{d}
P	271.34 ± 1.61^{a}	283.15 ± 1.61^{b}	286.11 ± 0.53^{c}	290.72 ± 1.17^{d}
K	416.20 ± 1.91^{a}	426.72 ± 1.36^{b}	433.72 ± 0.73^{c}	444.09 ± 1.58^{d}
Mg	124.33 ± 1.27^{a}	131.60 ± 1.89^{b}	136.30 ± 0.89^{c}	141.99 ± 2.16^{d}
Na	9.88 ± 1.49^{a}	14.47 ± 0.99^{b}	16.83 ± 0.87^{c}	18.43 ± 0.78^{d}
Micro-minerals				
Cu	0.46 ± 0.12^{a}	0.48 ± 0.26^{b}	0.60± 0.07^{b}	0.84 ± 0.09^{c}
Zn	2.12 ± 0.12^{a}	2.98 ± 0.27^{b}	3.63 ± 0.10^{c}	3.75 ± 0.22^{d}
Fe	4.26 ± 1.07^{a}	6.21 ± 1.33^{b}	6.67 ± 0.54^{b}	7.81 ± 0.29^{c}
Mn	6.21 ± 0.52^{a}	5.93 ± 1.12^{a}	5.90 ± 0.74^{a}	5.95 ± 0.54^{a}
DBFMF				
Macro-minerals				
Ca	308.66 ± 3.06^{a}	320.64 ± 2.35^{b}	325.85 ± 1.70^{c}	331.98 ± 1.36^{d}
P	315.77 ± 0.52^{a}	325.55 ± 0.77^{b}	338.57 ± 0.70^{c}	350.58 ± 0.87^{d}
K	360.92 ± 0.84^{a}	379.64 ± 0.44^{b}	392.68 ± 0.74^{c}	401.90 ± 1.55^{d}
Mg	144.31 ± 1.27^{a}	153.07 ± 0.17^{b}	164.61 ± 0.82^{c}	171.11 ± 1.89^{d}
Na	5.66 ± 1.73^{a}	10.94 ± 0.68^{b}	13.36 ± 1.66^{c}	14.83 ± 1.12^{c}
Micro-minerals				
Cu	0.44 ± 0.11^{a}	0.44 ± 0.05^{a}	0.67 ± 0.10^{b}	1.17 ± 0.08^{c}
Zn	1.64 ± 0.05^{b}	1.45 ± 0.18^{a}	1.45 ± 0.28^{a}	1.44 ± 0.11^{a}
Fe	5.07 ± 0.35^{a}	5.69 ± 0.59^{a}	5.28 ± 1.01^{a}	7.98 ± 0.33^{d}
Mn	16.62 ± 1.15^{b}	16.49 ± 0.32^{b}	13.60 ± 0.70^{a}	13.34 ± 1.02^{a}

Values are expressed by mean ± standard deviation, n = 3. Different superscripts in a row show significant different ($p < 0.05$). LBFMF = light brown finger millet flour; DBFMF = dark brown finger millet flour. Ca = calcium, P= phosphorus, K = potassium, Mg = Magnesium, Na = Sodium, Cu = copper, Zn = zinc, Fe = iron, Mn = manganese.

3.3.2 Functional Properties of Spontaneously Fermented Finger Millet Flours

Table 3.2 presents the functional properties of spontaneously fermented FM flours. The loose bulk density of both FM flours was within the range of 0.47 to 0.56 g/mL. Statistically, SF had no impact on loose bulk density of FM flours. The value of 0.47 g/mL is similar to that found by Elkhalifa et al. [43], for fermented sorghum flour. For PBD, a slight decrease with increasing period of SF was observed in both FM flours. The declining trend could be due to the SF of FM grains, which often results in the breakdown of proteins and carbohydrates into small units [30]. The lower bulk density of fermented FM flour helps with the formulation of reduced bulk weaning foods for babies [44].

Table 3.2. Functional properties of spontaneously fermented finger millet flours.

Fermentation period (h)	**LBD (g/g)**	**PBD (g/g)**	**WAC (g/g)**	**OAC (g/g)**	**SC (mL)**
LBFMF					
0	0.55 ± 0.07^{a}	0.79 ± 0.04^{a}	1.96 ± 0.10^{a}	1.20 ± 0.28^{a}	14.33 ± 0.58^{a}
24	0.56 ± 0.00^{a}	0.76 ± 0.01^{ab}	2.05 ± 0.06^{ab}	1.28 ± 0.09^{b}	14.00 ± 1.00^{a}
48	0.56 ± 0.03^{a}	0.75 ± 0.02^{ab}	2.07 ± 0.01^{ab}	1.36 ± 0.15^{b}	14.00 ± 1.00^{a}
72	0.52 ± 0.02^{a}	0.73 ± 0.03^{b}	2.10 ± 0.03^{b}	1.44 ± 0.17^{c}	13.33 ± 0.58^{b}
DBFMF					
0	0.47 ± 0.04^{a}	0.77 ± 0.00^{b}	2.05 ± 0.08^{a}	1.24 ± 0.15^{a}	14.00 ± 0.00^{a}
24	0.50 ± 0.03^{a}	0.74 ± 0.03^{ab}	2.06 ± 0.01^{a}	1.30 ± 0.10^{b}	13.67 ± 0.58^{a}
48	0.52 ± 0.02^{a}	0.74 ± 0.02^{ab}	2.08 ± 0.01^{a}	1.35 ± 0.20^{c}	13.67 ± 0.57^{a}
72	0.49 ± 0.04^{a}	0.71 ± 0.00^{a}	2.14 ± 0.05^{b}	1.39 ± 0.11^{d}	13.33 ± 0.58^{b}

Values are expressed by mean ± standard deviation, n = 3. Different letters in the column show significant different ($p < 0.05$). LBD, loose bulk density; PBD, packed bulk density; WAC, water absorption capacity; OAC, oil absorption capacity; SC, swelling capacity; LBFMF, light brown finger millet flour; DBFMF, dark brown finger millet flour.

The WAC is a measurement of how flour and water interact in a variety of foods [45]. The presence of hydrophilic groups which bind water in flour determines the ability of that flour to absorb water [35]. The WAC values in 72 h FM flours were significantly higher ($p < 0.05$) than those in other samples. This could be due to the higher presence of polar amino acids and hydrophilicity of carbohydrates [46] and a loss of starch polymers caused by SF resulting in 72 h FM flours possessing high WAC [30]. Igbabul et al. [32] argued that high WAC is good for making bakery products. Furthermore, a low WAC obtained in native FM flours suggested a less presence of carbohydrates and low water-binding hydrophilic groups. Adebowale et al. [47] stated that a lower WAC value suggests starch polymer structural compactness. Tenagashaw et al. [48] noted that a lower WAC of flour is good for producing thin gruels for baby formulas [48]. Awuchi et al. [25] found that the flour's WAC is important for baking, product homogeneity, and bulking.

The OAC of both FM flours increased as SF time increased and the highest OAC was obtained in 72 h fermented flours, while the lowest OAC was obtained in native FM flours. The increasing trend of OAC observed suggest that fermented FM flours may have contained more hydrophobic proteins which have better lipid binding properties, hence the increase of OAC was observed in fermented FM flours. Protein is the most important chemical component affecting OAC, and the chains of amino acids (alanine, leucine, etc.) that are non-polar interact with chains of hydrocarbon lipid [49]. The OAC increment of fermented flours observed was in consistent with the study of Adebiyi et al. [30], who noted an increment of OAC in pearl millet flour due to fermentation. A higher OAC observed means fermented FM flours have the potential to be used in food preparation because such quality retains flavour and improves mouth feel [50].

Ayo-Omogie and Ogunsakin [51] argued that the SC of flour is a crucial parameter that influence the consistency of the flour and it is impacted by the compositional structure of the flour. The SC of both FM flours ranged between 13.33 and 14.33 mL. A higher SC was obtained in native (0 h) FM flour, while lower SC was obtained in 72 h fermented FM flours. The SC of 72 h FM flour was reduced by SF and these findings were like those obtained by Adebowale and Maliki [52]. In their study, the SC of flour decreased after SF. Olukomaiya et al. [53] reported that a higher SC indicates an enhancement of flour functionality, which would result in a good food product in the end. Ayo-Omogie and Ogunsakin [51] mentioned that a lower SC suggests that fermented FM flour can produce a nutritionally dense food for infants.

3.3.3 Viscosity of Spontaneously Fermented Finger Millet Flours

The viscosity of spontaneously fermented FM flours is shown in Figure 3.1. According to the cold paste results, there was no significant differences ($p < 0.05$) observed among the flour samples. The cooked paste viscosity for native FM flour was significantly higher than that of spontaneously fermented FM flours. Uvere et al. [54] noted that the decreasing trend observed in fermented flours might be attributed to starch granule hydrolysis due to amylases activity during SF. Many studies have also found that starch hydrolysis' tendency to form sugars results in reduced viscosity of flour samples in various food processing technologies [32,44,11,55]. Uyere et al. [54] stated that the decrease of viscosity in fermented FM flours could also potentially be owing to granule structural degradation caused by milling, resulting in an increase of amount of soluble materials such as dextrin with short chain and some sugars. Usman et al. [56] stated that the low viscosity of fermented FM flours indicates that the flour is suitable for making weaning foods for new-borns. Both cold and cooked paste values obtained in native FM flour were similar to that obtained by Ramashia [57] and Pawase et al. [58] for fortified and unfortified FM flours. These findings were also in consistent with Onweluzo and Nwabugwu [33], who studied how SF affect the viscosity of pearl millet flour.

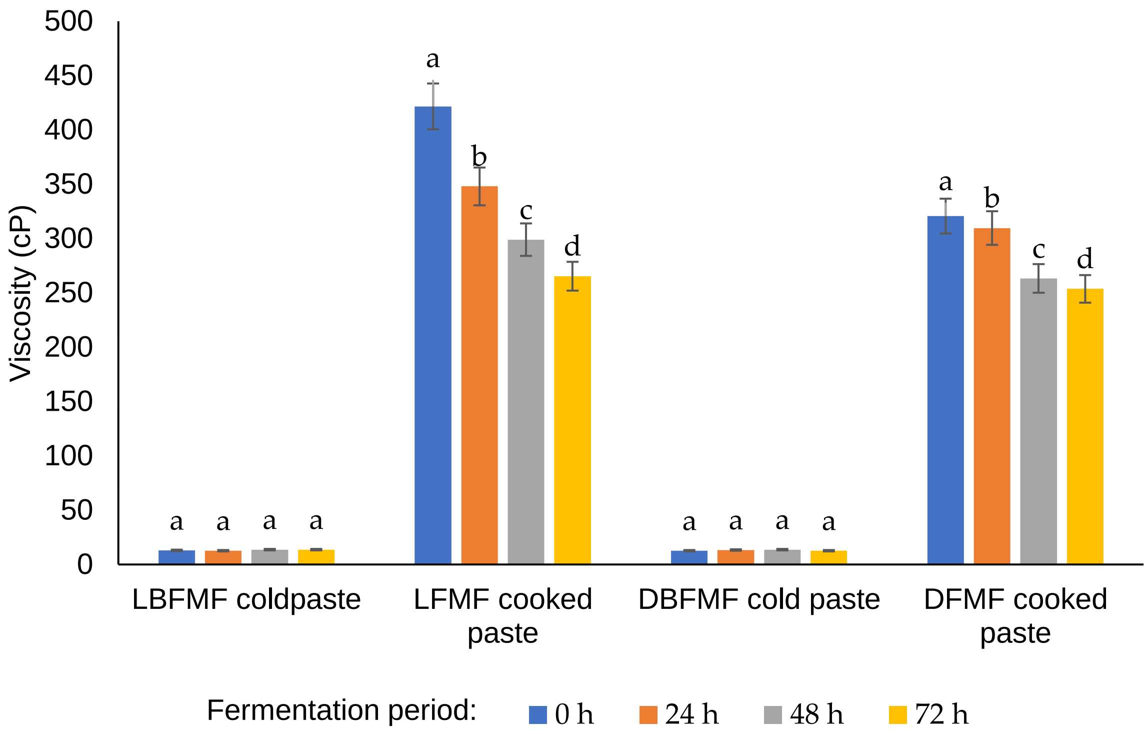


Figure 3.1. Influence of spontaneous fermentation on cold paste and cooked paste of finger millet flours. Different letters show statistically different effects ($p < 0.05$). LBFMF= light brown finger millet flour; DBFMF= dark brown finger millet flour.

3.3.4 Thermal Properties of Spontaneously Fermented Finger Millet Flours

Table 3.3 summarises the impact of SF on the thermal properties of FM flours. The gelatinisation temperatures [onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c)] of both FM flours increased with the increasing period of SF. Higher gelatinisation temperatures were observed in 72 h fermented flours. The increase in peak temperature after fermentation might be attributed to accumulation of proteolytic enzymes generated by natural bacteria that break down the cell walls of grain, resulting in more starch released and higher crystallite structures proportions in the sample [38]. Kumoro and Hadyiyat [59] stated that SF process can alter the macromolecular structure or arrangement of amylopectin and amylose in flour granules, changing gelatinisation temperatures.

A similar increase of onset temperature (T_o) and peak temperature (T_p) after fermentation was observed by Amadou et al. [16], Kumoro and Hadiyat [59] and Olamiti et al. [60] for foxtail millet, Korean wheat and pearl millet flours. The inherent modifications of granule size, morphology, starch distribution, and internal starch fraction organisation inside the granules could explain the disparities in To, Tp, and Tc values identified in fermented FM flours [61].

Table 3.3: Influence of spontaneous fermentation on the thermal properties of finger millet flours

Fermentation period (h)	T_o **(°C)**	T_p **(°C)**	T_c **(°C)**	T_r **(°C)**	**∆H(J/g)**
LBFMF					
0	76.64 ± 1.44^{a}	78.41 ± 1.16^{a}	79.99 ± 1.50^{a}	3.35 ± 1.60^{a}	5.07 ± 0.79^{ab}
24	79.24 ± 0.81^{b}	80.97 ± 0.82^{b}	82.25 ± 1.48^{b}	3.01 ± 0.67^{a}	4.30 ± 0.54^{b}
48	81.12 ± 1.25^{bc}	82.06 ± 0.97^{c}	85.01 ± 1.29^{c}	3.88 ± 1.22^{a}	4.28 ± 1.20^{ab}
72	82.47 ± 0.89^{c}	84.48 ± 4.58^{d}	86.39 ± 0.56^{d}	3.92 ± 1.43^{a}	2.99 ± 0.22^{a}
DBFMF					
0	69.42 ± 1.00^{a}	70.98 ± 0.93^{a}	78.24 ± 2.95^{a}	8.82 ± 1.96^{a}	4.87 ± 1.92^{c}
24	70.10 ± 0.25^{b}	72.21 ± 1.04^{ab}	78.89 ± 1.67^{a}	8.79 ± 1.43^{a}	4.50 ± 0.84^{b}
48	71.03 ± 0.60^{c}	73.62 ± 1.87^{bc}	79.95 ± 1.20^{b}	8.92 ± 0.66^{a}	4.38 ± 1.06^{ab}
72	72.04 ± 1.05^{d}	75.67 ± 0.68^{c}	81.63 ± 0.80^{c}	9.59 ± 0.61^{a}	3.85 ± 0.85^{a}

Values are expressed by mean ± standard deviation, n = 3. Different superscripts in the column show significant different ($p < 0.05$). T_o = onset temperature; T_p = peak temperature; T_c = conclusion temperature; T_r = gelatinisation temperature range; ∆H= gelatinisation enthalpy; LBFMF = light brown finger millet flours; DBFMF = dark brown finger millet flours.

High gelatinisation temperatures obtained in 72 h fermented FM flours suggested that more energy was required to start the starch gelatinisation process. The increase in gelatinisation temperatures of fermented flours could be due to the production of amino acids resulting from protein alteration during SF [8]. However, starches with low gelatinisation temperatures, such as those found in native and 24 h fermented flours were best renowned for excellent cooking quality [62]. In terms of gelatinisation temperature range (T_r), there was no significant different observed among the samples. The gelatinisation enthalpy (∆H) of both FM flours decreased as SF time increased. This decrease means that fermented flours would require less energy to disrupt starch granules bonds [63]. A similar decreasing trend of ∆H was also observed by Ahmed et al. [64] and Bian et al. [65], for fermented koreeb seed flours and proso millet flour.

3.3.5 Pasting Properties of Spontaneously Fermented Finger Millet Flours

Pasting properties of FM flours are used to evaluate if the flour is suitable for use in baking [57]. They also reflect the variations in flour viscosity that occur when it is heated in excess water while being constantly stirred [65]. Table 3.4 summarises the effect of SF on pasting properties of FM flours. The peak viscosity (PV) decreased with the increasing period of SF and later started to increase at 72 h. The PV indicates the maximum SC of starch granules and WAC [31]. The higher PV observed in native FM flour was probably due to the flour having a higher content of starch [66]. The lower PV indicates that the fermented FM flours have lower thickening power. The shorter starch chains formed during SF could be responsible for the decrease in PV [67]. The PV values obtained in native flour were similar to those obtained by Dasa and Binh [68] for a variety of millet flour.

The trough viscosity (TV) obtained from native flour was higher than that obtained from fermented FM flours. The TV reflects the gel or viscous paste forming capacity of the flour after heat treatment and how well it can withstand stress generated by stirring Adegunwa et al. [69]. Therefore, the lower TV observed in fermented flours suggests the shear resistance of swollen granules.

The breakdown viscosity (BDV) value determines how easily swelling granules can be dissolved, and this indicates the flour product's stability [70]. It is also the differences between peak viscosity and through viscosity. The BDV of light brown FM flour increased with an increasing period of SF up to 48 h period and started to decrease at 72 h. In dark brown FM flour, the BDV increased with an increasing period of SF. The increment in BDV of fermented flours as compared to the native flours could be linked to the increase of protein content due to SF [18, 71]. Similarly, Geng et al. [72] found an increase of BDV after the fermentation of rice. The higher BDV viscosity, the poor the sample's capacity to endure heat and shear stress generated by cooking [73, 74]. The lower BDV of both native FM flours indicates the starches' resistance to thermal treatment and shearing.

The final viscosity (FV) values of native FM flours were higher than that of fermented FM flours. The decrease of FV observed in fermented flours could be ascribed to the breakdown of amylose into sugars during SF [75]. Lower FV values observed in fermented FM flour denotes a loss of capacity of flours to make a viscous paste. According to Oloyede et al. [74], FV represents the starch's capacity to form a gel and paste in the flour after heat treatment.

The setback viscosity (SV) is a retrogradation index that is linked to the amylose content [76]. The higher setback value, the more likely amylose is to retrograde and form a gel structure [75]. The findings in this study show that SF increased the SV values of FM flours, and this could be due to amylose's ability to rearrange itself after being disrupted. A similar increase of SV values after fermentation was found by Said et al. [67] for rice flour. The high values of SV obtained in fermented flours imply that the flours could be good for making noodles and jelly foods [77].

Table 3.4: Pasting Properties of spontaneously fermented finger millet flours.

Fermentation period (h)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time	PT (°C)
LBFMF							
0	2410.67 ± 48.23^{a}	2308.67 ± 53.59^{a}	102.00 ± 7.94^{c}	2414.00 ± 66.84^{a}	105.33 ± 18.01^{d}	5.11 ± 0.75^{c}	74.82 ± 0.45^{a}
24	2111.67 ± 24.01^{c}	1974.33 ± 35.59^{b}	137.33 ± 12.34^{b}	2181.00 ± 15.72^{b}	206.67 ± 20.60^{c}	5.24 ± 0.10^{c}	75.05 ± 0.05^{b}
48	1709.67 ± 7.23^{d}	1349.67 ± 12.05^{d}	360.00 ± 18.73^{a}	1616.33 ± 7.51^{c}	266.67 ± 7.37^{b}	6.00 ± 0.13^{b}	75.13 ± 0.32^{b}
72	2246.67 ± 12.58^{b}	1889.67 ± 4.73^{c}	357.00 ± 17.35^{a}	2239.00 ± 11.36^{b}	349.33 ± 16.07^{a}	6.69 ± 0.08^{a}	75.25 ± 0.43^{b}
DBFMF							
0	2876.67 ± 30.99^{a}	2739.67 ± 32.01^{a}	137.00 ± 2.73^{d}	2959.00 ± 34.66^{a}	219.33 ± 4.26^{b}	6.07 ± 0.07^{c}	75.02 ± 0.02^{a}
24	2776.00 ± 25.51^{b}	2652.33 ± 33.08^{b}	123.67 ± 14.19^{c}	2871.33 ± 32.96^{b}	219.00 ± 7.81^{b}	6.13 ± 0.07^{c}	75.28 ± 0.23^{b}
48	2762.00 ± 15.72^{b}	2578.67 ± 18.58^{c}	183.33 ± 33.62^{b}	2874.67 ± 27.15^{b}	296.00 ± 45.43^{a}	6.40 ± 0.00^{b}	75.42 ± 0.52^{c}
72	2695.67 ± 11.59^{c}	2480.33 ± 10.69^{d}	215.33 ± 2.88^{a}	2754.67 ± 10.97^{c}	274.33 ± 7.57^{a}	6.80 ± 0.07^{a}	75.73 ± 0.43^{d}

Values are expressed by mean ± standard deviation, n = 3. Different letters in the column show significant different ($p < 0.05$). LBFMF = light brown finger millet flour; DBFMF = dark brown finger millet flour.

Peak time determines cooking time [78]. The highest peak time in this study was found in 72 h fermented FM flours, while the lowest peak time was found in native flours. This result suggested that 72 h fermented FM flours require more cooking time than native flours. Oyeyinka et al. [79] observed a similar increase of peak time values as fermentation progressed. Other studies have reported similar values of peak time [80, 81].

3.3.5 Colour Attributes of Finger Millet Flours

The colour of light and dark brown FM flours was measured in terms of L*, a* and b* values as shown in Table 3.5. The L* values of the two flours increased with increasing period of fermentation. A higher L* value was obtained in 72 h fermented flours while a lower L* value was obtained in native flours.

Table 3.5. Effect of fermentation on the colour properties of spontaneously fermented finger millet flours.

Fermentation period (h)	L*	a*	b*	Chroma	Hue angle	ΔE
Light brown FM flours						
0	75.05 ± 0.21^{a}	4.28 ± 0.21^{c}	7.32 ± 0.05^{d}	8.48 ± 0.03^{b}	59.67 ± 0.25^{a}	0.00 ± 0.00^{a}
24	78.04 ± 0.34^{b}	3.32 ± 0.34^{b}	6.72 ± 0.09^{a}	7.50 ± 0.09^{a}	63.70 ± 0.38^{b}	3.19 ± 0.35^{b}
48	78.75 ± 0.27^{c}	3.26 ± 0.27^{b}	6.85 ± 0.07^{b}	7.58 ± 0.06^{a}	64.58 ± 0.38^{c}	3.86 ± 0.28^{c}
72	83.24 ± 0.09^{d}	2.62 ± 0.09^{a}	7.00 ± 0.03^{c}	7.48 ± 0.07^{a}	69.46 ± 0.03^{d}	8.35 ± 0.08^{d}
Dark brown FM flours						
0	72.07 ± 0.25^{a}	3.54 ± 0.03^{c}	8.36 ± 0.04^{d}	9.07 ± 0.02^{d}	67.03 ± 0.26^{a}	0.00 ± 0.00^{a}
24	74.56 ± 0.26^{b}	2.99 ± 0.06^{b}	7.95 ± 0.07^{c}	8.49 ± 0.08^{c}	69.36 ± 0.33^{b}	2.59 ± 0.27^{b}
48	74.83 ± 0.35^{b}	3.04 ± 0.01^{b}	8.10 ± 0.05^{b}	8.65 ± 0.04^{b}	69.48 ± 0.14^{b}	2.82 ± 0.34^{b}
72	76.08 ± 0.42^{c}	2.79 ± 0.04^{a}	7.82 ± 0.06^{a}	8.30 ± 0.06^{a}	70.34 ± 0.41^{c}	4.11 ± 0.40^{c}

Mean ± standard deviation. Different letters in the columns show significant different ($p < 0.05$) of mean values. L* =lightness; a* = redness; and b* = yellowness. FM = Finger millet.

The increase in the lightness of flour samples could be due to dissolution of coloured (red or yellow) pigments caused by changes in the carbohydrates or protein hydrolysate during fermentation [82]. Akinola et al. [83], also showed an increase in lightness of pearl millet flour caused by fermentation. The results of this study were also similar to those obtained by Siroha et al. [84] and Ramashia [56] for different cultivars of FM flours.

In terms a* values, a higher a* values were obtained in native light and dark brown FM flours while lower a* values was obtained in 72 h fermented FM flours. This means that both native light and dark brown FM flours contained higher red pigmentation. In both cultivars, however, there was no significance difference ($p < 0.05$) between 24 and 48 h fermented FM flours. In terms of a* values, the findings in this investigation were similar to those of Olamiti et al. [60] for malted and fermented pearl millet flours. The b* values were higher in both native FM flours and lower in 72 h fermented flours. Positive b* values obtained in this study showed yellow pigmentation of both FM flours. Similar results of b* values were also obtained by Siroha et al. [84] for millet flours. According to a* and b* results, fermented flours were less red and yellow as com-pared to the native flours.

The chroma values were higher in native flours than in fermented flours. The hue angle (H°) values of the two flours increased significantly with increasing period of fermentation. According to Mudau et al. [71], yellow, green, and blue hues are represented by angles of 90°, 180°, and 270°, respectively, whereas red hue is represented by a hue angle of 0° or 360°. Therefore, all the flour samples were closer to red. The colour difference (ΔE) values of 72 h fermented flours as compared natives were significantly higher, indicating that fermentation changed the colour of flours.

3.3.6 *Fourier Transmission-Infrared of Spontaneously Fermented Finger Millet Flours.*

Figure 3.2 show the FTIR spectra regions (O – H stretch region, C – H stretch region, and fingerprint region) of light and dark brown FM flours. The O – H region of light brown FM flours showed bands (peaks) within the range of 3273.45 to 3273.92 cm-1. In dark brown FM flours, the absorption peaks were in the range of 3274.25 to 3272.74 cm-1. The observed peaks could be ascribed to the stretching vibrations of O –H and differences in peaks could be linked to variations of moisture content of FM flours caused by fermentation as reported by Mudau et al. [71]. Moisture content have been reported to be nutrient responsible for the peaks in O – H stretch region [28, 84]. Higher peak values observed in fermented flours suggest the presence of more alcohol produced during fermentation.

The C – H band's width increased in the two flours as fermentation period increased, with absorption peaks ranging from 2924.29 to 2925.35 cm^{-1} and 2924.47 to 2925.49 cm^{-1}, respectively. This was probably caused by the stretching vibrations of aromantic and aliphatic C-H bonds [30]. The variation in the peaks could be correlated to differences observed in the fat content of fermented FM flours as reported by Mudau et al. [71]. They were also peaks within the range of 1644.55 to 1651.86 cm^{-1}, which denote the presence of amide I (stretching and bending vibrations of C = O).

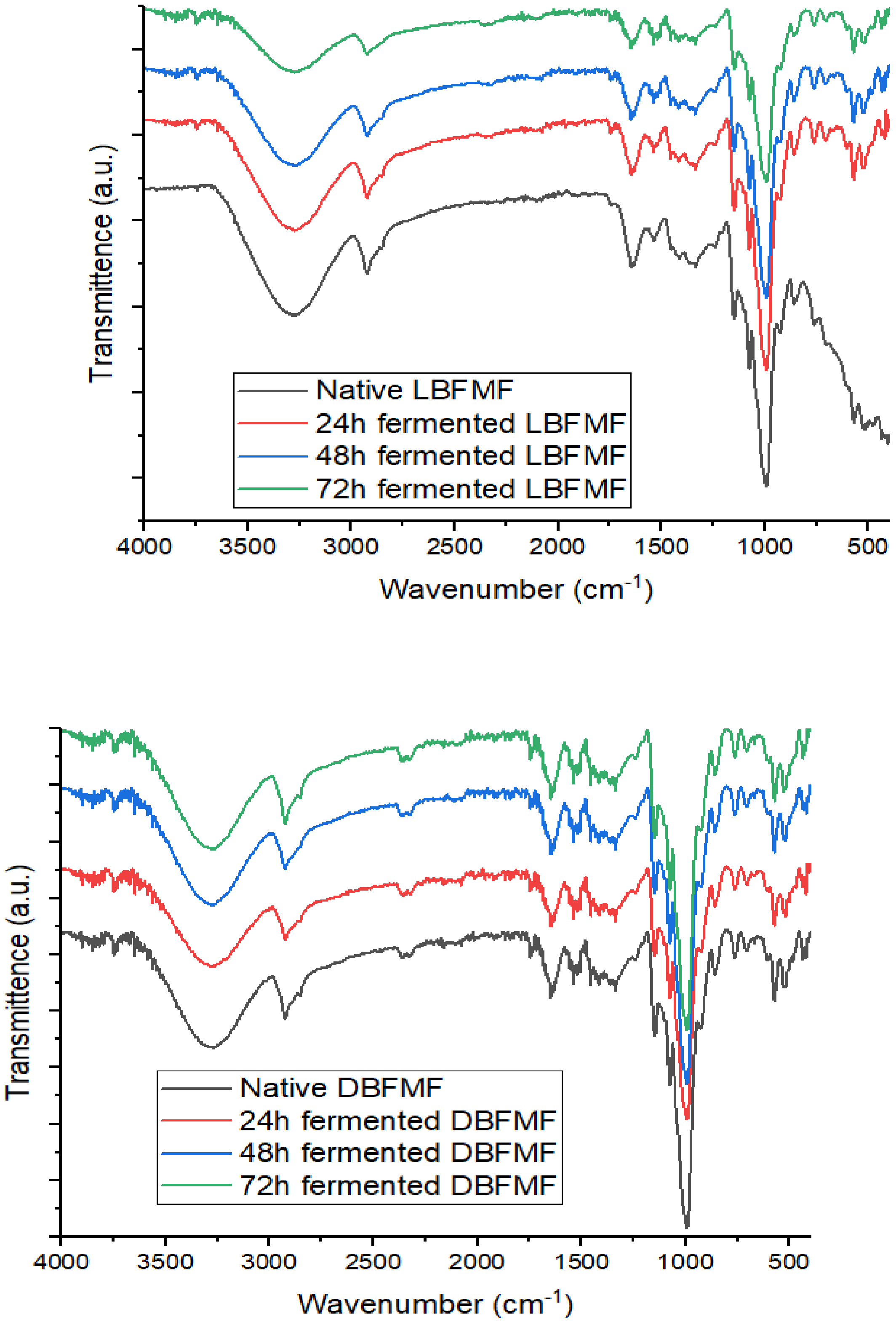
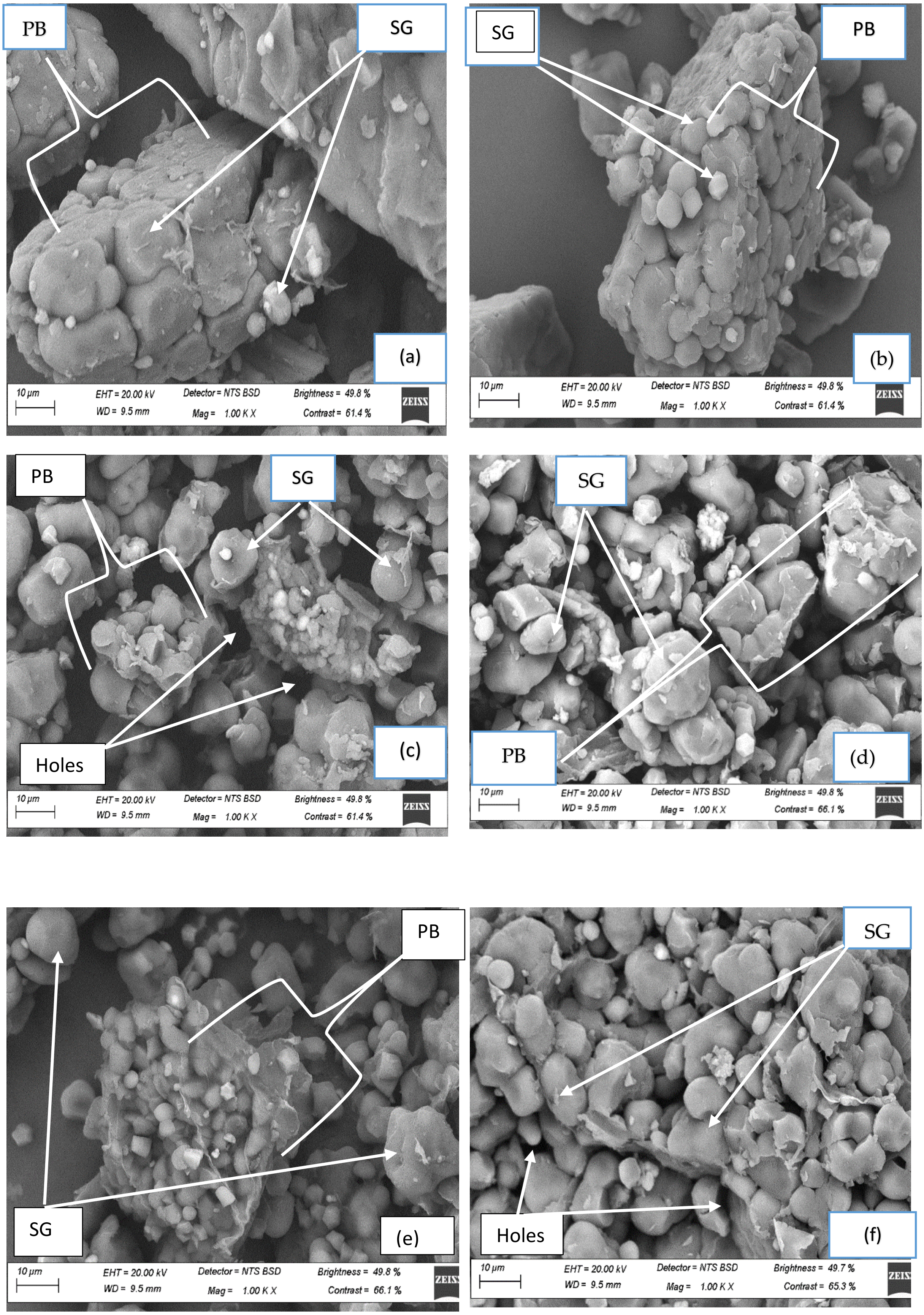


Figure 3.2. FTIR spectra of finger millet flours. LBFMF = Light brown fermented finger millet flours, DBFMF = Dark brown fermented finger millet flours.

The stronger amide I peaks intensities in the fermented flours could be linked to the increment of protein content caused by fermentation of FM flours as observed by Mudau et al. [71]. Series of bands in the fingerprint region (1500-900 cm-1) were also observed in this study. The peaks at around 1338.26 in native and fermented flours show that the flours contained O–C–H, C–C–H and C–O–H [86]. Other notably range of peaks in the FM region were observed near 1149.40 to 1148.02 cm-1, 1076.40 to 1076.20 cm-1, and 994.33 to 993.10 cm-1 which show that functional groups including C–O, C=O, and C–H were present in FM flours, as similarly reported by Navyashree et al. [82]. The disparities in peaks between 1148.85 and 994.25 994.25 cm-1 could be attributable to the stretching and bending vibrations of C–H, C–O–C and C–O bonds [87], and the differences in carbohydrate and protein contents caused by different periods of fermentation [70]. Other absorbance peaks ranging from 859.69 to 406.94 cm-1 denote the presence of COH, CCH, OCH in the flours [86] and the variations among the peaks could be linked to changes in the protein content of flours caused by fermentation [71]. Adebiyi et al. [30], also linked the disparities of peaks to protein changes due to fermentation.

3.3.6 Scanning Electron Microscopy of Spontaneously Fermented Finger Millet Flours

Figure 3.3 shows the scanning electron micrographs of fermented FM flours. The native light brown FM flour showed a large compact structure of protein bodies (PB) which was broken down during SF. The starch granules (SG) in both native flours varied from small to large and were attached to each other in PB. The subsequent fermented FM flours showed small PB, and more liberated SG compared to the native flours that had large structure of PB with entrapped SG. This could be due to the SF that breaks down large complex compound into simple molecules. The liberated SG in the fermented flours varied from small to large with round and oval shapes. There were also more holes observed in between the SG of fermented flours compared to the native flours. Narayanasamy [1] also found pits in fermented samples and attributed this to the enzymatic degradation of SG by microorganism during SF. The total liberation of SG was observed in both 72 h fermented FM flours, and this was probably due to the breaking of cell walls caused by SF. Salmenkallio-Marttila et al. [88] indicated that the breakdown of cell wall has an impact on the microstructure of starch granules. Nainggolan et al. [89] observed similar results of SG for fermented cassava flour. Many studies have also linked microstructural alterations to fermentation [90, 91, 92]. Changes or loosening of starch structure observed in FM flours due to SF could be the reason for the observed disparities in the thermal properties, SC, WAC, and AOC of the flour. Khoza et al. [93] stated that there is a relationship between the morphology of flour and SC, WAC as well as thermal properties of the flour.


PB
SG
(a)
10 µm
EHT = 20.00 kV
WD = 9.5 mm
Detector = NTS BSD
Mag = 1.00 K X
Brightness = 49.8 %
Contrast = 61.4 %
ZEISS
SG
PB
(b)
10 µm
EHT = 20.00 kV
WD = 9.5 mm
Detector = NTS BSD
Mag = 1.00 K X
Brightness = 49.8 %
Contrast = 61.4 %
ZEISS
PB
SG
Holes
(c)
10 µm
EHT = 20.00 kV
WD = 9.5 mm
Detector = NTS BSD
Mag = 1.00 K X
Brightness = 49.8 %
Contrast = 61.4 %
ZEISS
SG
PB
(d)
10 µm
EHT = 20.00 kV
WD = 9.5 mm
Detector = NTS BSD
Mag = 1.00 K X
Brightness = 49.8 %
Contrast = 66.1 %
ZEISS
PB
SG
(e)
10 µm
EHT = 20.00 kV
WD = 9.5 mm
Detector = NTS BSD
Mag = 1.00 K X
Brightness = 49.8 %
Contrast = 66.1 %
SG
Holes
(f)
10 µm
EHT = 20.00 kV
WD = 9.5 mm
Detector = NTS BSD
Mag = 1.00 K X
Brightness = 49.7 %
Contrast = 65.3 %

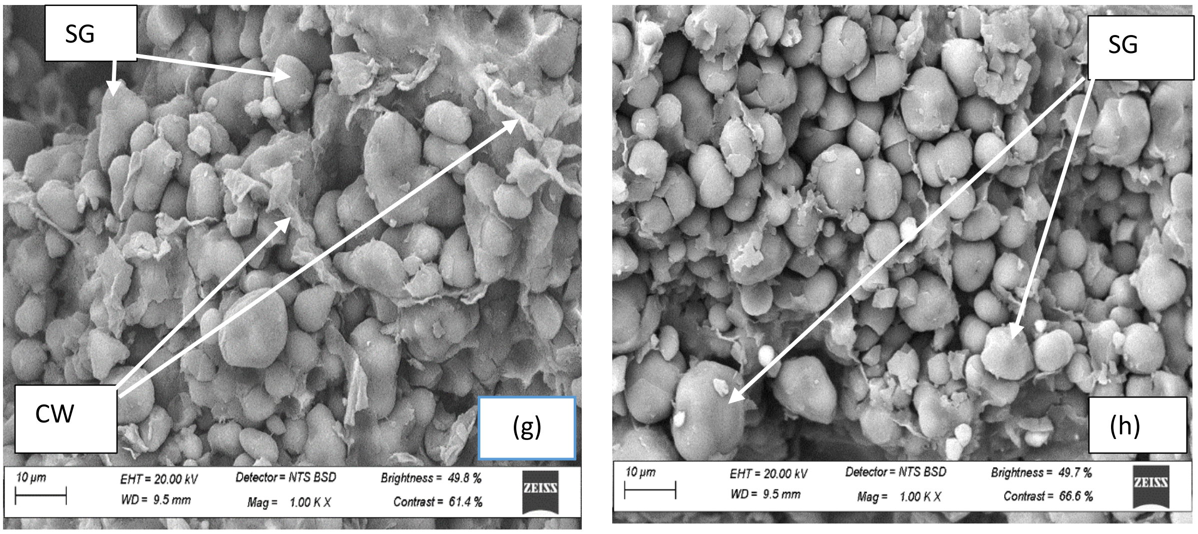


Figure 3.3: Scanning electron micrographs of fermented light and dark brown FMF; (a) native light brown FMF; (b) native dark brown FMF; (c) 24 h fermented light brown finger millet; (d) 24 h fermented dark brown FMF; (e) 48 h light brown FMF; (f) 48 h fermented dark brown FMF; (g) 72 h fermented light brown FMF; (h) 72 h fermented dark brown FMF; PB = protein bodies; SG = Starch granules; CW= cell walls; FMF = finger millet flours.

3.4 Conclusion

In this study, SF positively influenced the functional and thermo-pasting properties, as well as the mineral content of both cultivars of FM flours. Spontaneous Fermentation has shown to be an effective treatment in reducing packed bulk density, swelling capacity, cooked paste viscosity, peak viscosity, and trough viscosity of FM flours, which would make it suitable to produce weaning foods or nutritionally dense foods. The improved water/oil absorption capacity, setback viscosity, and pasting temperature of fermented FM flours particularly 72 h, make the flours good for utilisation during manufacturing of bakery products, jelly foods and thickening food. The superiority of 72 h fermented flours in terms of minerals, suggest that flours should be used to make foods that addresses mineral deficiencies in children, celiac and elderly individuals. Fermentation also modified the microstructural properties of FM flours. Future studies should focus on developing new gluten-free food products from fermented FM flours for people who suffer from celiac diseases.

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CHAPTER 4: NUTRITIONAL QUALITY, ANTIOXIDANT, MICROSTRUCTURAL AND SENSORY PROPERTIES OF SPONTANEOUSLY FERMENTED GLUTEN-FREE FINGER MILLET BISCUITS

Abstract

Finger millet (FM) is a nutritious and gluten-free cereal grain which is rich in dietary fibre, minerals and antioxidant properties thereby making it an ideal raw material for preparing gluten-free foods for people suffering from celiac disease. Spontaneous fermentation of FM grains has shown improved nutritional and functional properties of its flour and can be used as a functional ingredient for gluten free biscuits. The aim of this study was to determine the effect of spontaneous fermentation (SF) on the nutritional quality, antioxidant, microstructural, and sensory characteristics of gluten-free FM biscuits obtained from light and dark brown FM flours. Results showed that SF decreased ash, crude fibre, and crude fat contents as well as total phenolic and flavonoids contents. Protein content, carbohydrates content, energy values, antioxidant activity (DPPH and FRAP), and mineral content of FM biscuits increased due to SF. The colour properties such as lightness (L*), showed a significant increase as SF period increased in light brown FM biscuits, however dark brown FM biscuits showed no significant difference. The Hue angle and colour differences (ΔE) of FM biscuits increased with increasing period of SF ranging from 43.20 to 53.76° and from 0.67 to 7.96, respectively. Spontaneous fermentation also decreased physical properties of biscuits such as diameter (4.76 to 4.54 cm), weight (12.77 to 11.99 g), spread ratio (7.25 to 6.05) while an increase in thickness and hardness was noted. Spontaneous fermentation also induced changes on the microstructure of FM biscuits. Among the fermented biscuits, panellists preferred 24 h gluten-free fermented FM biscuits since they had better sensory properties. Overall, SF enhanced the nutritional value and health promoting compounds of gluten-free FM biscuits.

Keywords: Fermentation, millet, proximate composition, polyphenols, organoleptic properties.

4.1. Introduction

Millets are cereals that belong to the Poaceae family of grasses. The name millet is derived from “mille”, a French word meaning a thousand, which signifies that thousands of grains can be found in a handful of millet [1]. Millets come in different varieties, including finger millet, pearl millet, little millet, barnyard millet, and kodo millet, also known as *Eleusine coracana*, *Pennisetum glaucum*, *Panicum sumatrense*, *Echinochloa Frumentacea* Link, and *Setara italic*, respectively [2,3]. Finger millet (FM), after sorghum, pearl millet, and foxtail millet, is the fourth most important millet in the world [4]. It is commonly grown in Africa and South Asia, and accounts for around 10% of the world's 30 million tons of millet production and this shows that the production of FM is sufficient for market application [5]. It is also a nutrient-dense grain, which is underutilised, neglected, and is commonly known as African millet, ragi in India and mufhoho in Venda, South Africa. Protein, dietary fibre, minerals, polyphenols, and antioxidants are all abundant in FM grains [6]. Finger millet is a rich source of calcium, potassium and manganese compared to other millets [7]. As compared to all cereals, FM contains the highest content of calcium [8]. The grains contain no gluten and have a very low glycaemic index, which is good for celiac patients as well as those with heart disease or hypertension [9,10].

Biscuits are an ideal flour-based food that can be consumed as snack food by young and elderly people [11]. They have been utilised as a vehicle to deliver important nutrients (proteins and vitamins), polyphenols, and antioxidants [12,13]. They have advantages of being easily digestible and consumable. Among foods that are ready to eat, biscuits are significantly important because they are broadly accepted, cheap and their shelf life is quite long [14]. Unlike bread, biscuits are small and easy to bake without gluten and still have good sensory attributes that are appealing to consumers [11]. They are produced by mixing several ingredients, such as flour, sugar, water, and fat to form a dough [15].

There is a growing need for gluten-free (GF) products to tackle the issue of celiac disease. Celiac disease is a systemic, immune-mediated enteropathy, which is triggered by gluten protein in genetically susceptible individuals [16]. About 40-60 million people globally suffer from celiac disease and it is only treated by consuming GF food products [17]. However, nutrients deficiencies in GF food products are a concern because most GF products are commonly made from refined flours that lack protein, dietary fibres and micronutrients such as minerals and vitamins [18]. Finger millet is high in micronutrients; thus, it can be used as a potential material to make micronutrient-rich GF products to address nutritional challenges in GF diets [19,20]. With poor accessibility of minerals of plant-based diets, processing technologies such as spontaneous fermentation can be utilised to improve the bioavailability of micronutrients such as calcium, iron, and zinc as well polyphenols and antioxidants activity [21,22]. Research shows that spontaneous fermentation (SF) reduces antinutritional factors and enhance the bio-accessibility of nutrients [23,24]. Moreover, SF has been reported to improve protein content and dietary fibre in pearl millet bread and foxtail flour, respectively [25,26].

Lactic acid bacteria are some of the microorganisms that have been reported to bring desirable changes in FM flours [27]. Further, *Lactobacillus* and *Pediococcus* sp are used as fermenting microorganism in the production of Indian fermented FM food product (*Koozhu*) and this shows the safe use of fermented FM flour as an ideal substrate for the development of functional foods [28]. Modification in the microstructure, proximate composition, mineral composition, polyphenols, and sensory acceptance of pearl millet biscuits due to SF have also been reported [14,29,30]. However, little is known about how different periods of SF affects nutritional composition, polyphenols, antioxidant activity, physical, microstructural properties, and sensory acceptability of gluten-free FM biscuits. Hence, the objective of this study was to examine the nutritional quality, antioxidant, microstructural and sensory properties of spontaneously fermented gluten-free FM biscuits.

4.2 *Materials and Methods*

4.2.1 Materials

Two cultivars (light and brown) of FM grains as well as additional baking ingredients (sunflower oil, baking powder, vanilla essence, and sugar) for biscuits production were purchased from local street vendors and Shoprite supermarket in Thohoyandou, South Africa. All analytical grade reagents and standards (boric acid, quercetin standard, Folin-Ciocalteu reagent, 2,2-diphenyl-lpicrylhydrazyl (DPPH), gallic acid standard, trichloroacetic acid) used were procured from Merk chemicals (PTY) Ltd, Germiston, South Africa.

4.2.2 Finger Millet Flour Production

The grain chaffs and other unwanted materials were removed through winnowing, sorting, and screening. The grains were then washed with clean water and dried (40 °C) for 24 h using an air oven drier. After drying, some of grains were ground pulverised (Retsh ZM 200 Miller, Germany) to obtain native flour, while the remaining grains were kept for SF process. The native flour was sifted with a 500 µm sieve, placed in a polyethylene bag and refrigerated at 4 °C [31].

4.2.3 Finger Millet Grains Fermentation

Fine fermented FM flours were obtained using a modified method of Adebiyi et al. [14]. Approximately 400 g of FM grains were steeped in 1600 mL of distilled water in a closed container and left to spontaneously ferment at a temperature of 28 °C for 24, 48 and 72 h. After each fermentation period, the water was discarded, and the wet fermented grains were oven dried (40 °C) for 24 h. After that, the fermented grains were ground into (Retsh ZM 200 Miller, Germany) flours, which was sieved with 500 µm sieve to obtain fermented FM flours.

4.2.4 Biscuit Production

The FM biscuits were produced following a slightly modified method used by Serrem et al. [32]. The recipe for biscuits consisted of 112.5 g FM flour, sunflower oil (33 g), sugar (28 g), baking powder (0.75g), vanilla essence (6.75 g) and water (60 g) which were blended well in a bowl to form a dough, which was carefully rolled by hand to the desired thickness (0.7 cm) and cut with circular-shaped biscuit cutter (5.3 cm diameter). The moulded dough was then placed in an oven (Defy, Model DSS700, Midrand, South Africa) and baked (180 °C) for 18 min. Baked products were cooled down for 20 min at room temperature before being packed in a plastic and kept in a refrigerator until further analysis. The entire production of biscuits and analyses were replicated three times to validate the results, and nine biscuits per formulation were analysed. Biscuits made from native flours were used as control.

4.2.5 Proximate Composition of Fermented Finger Millet Flours and Biscuits

The AOAC [33] methods: 934.01, 978.10, 923.03, 920.39, and 990.03 were used to determine moisture, crude protein, ash, crude fat, and crude fibre of FM flours and biscuits. The carbohydrate percentage of the flour and biscuit samples was calculated using the formula:

Carbohydrate (g) = 100 – [protein (g) + moisture content (g) + lipid (g) + ash (g)+ crude fibre (g)]

The energy content of FM flours and biscuits was obtained by using the formula:

Energy content (kcal) = g/100g carbohydrate ×4 + g/100g fat ×9 + g/100g protein ×4 + g/100g fibre ×2

4.2.6 Mineral Analysis of Fermented Finger Millet Biscuits

The macro and micro-minerals of FM biscuits were assessed following a method used by Ramashia et al. [34]. Milled biscuits samples (2 g) were burned in the muffle furnace overnight at 550 °C to obtain ashes. The obtained ashes were treated with 50% HCL (10 mL) and 5 mL of 33% HNO_3 and the combination was allowed to stand for 1 h in the water bath. Afterward, HCL (10 mL) was added to the mixture and left in the water bath for 15 min, before it was transferred into 100 mL volumetric flask. Thereafter, distilled water was added into the 100 mL volumetric until the 100 mL mark was reached. The mixture was placed in the water bath for 1 h. Approximately 10 mL of HCL was added and left in a water bath for 15 min. The mixture was then added into 100 mL volumetric flask and filled to 100 mL mark with distilled water before it was well mixed. The inductively coupled argon plasm atomic emission spectroscopy was used to analyse the minerals and the obtained results were expressed as mg/100g.

4.2.7 Polyphenols and Antioxidant Activity of Fermented Finger Millet Biscuits

Extraction of polyphenols, DPPH and iron reducing power was determined by mixing 50 g each of flour with methanol (500 mL) for 24 h. Thereafter, the combination was subjected to centrifugation (Rotina 380 R- Labotech Ecotherm, South Africa) for 10 min at 3000 rpm, filtered using Whatman paper into beakers and transferred into different centrifuge tubes which were kept in the freezer for further analysis [35].

4.2.7.1 Total Phenolic Content

The TPC of FM biscuits extract was examined following a method employed by Demov et al. [36] with slight modifications. An extract (0.2 mL) was combined with a mixture of 5 times diluted Folin-Ciocalteu (2.5 mL) and 5 mL distilled water in test tubes. After 5 min, 15% sodium carbonate (7.5 mL) was added to the tubes, vortexed (Model 36110740, Separation Scientific, South Africa), and the mixture was stored in the dark for 30 min. A spectrophotometer (UV-1600, Shimadzu, Tokyo, Japan) was used to the record the values of absorbance at 760 nm. The standard curve was prepared with gallic acid and obtained results were expressed in milligrams of gallic acid per gram of biscuit sample.

4.2.7.2 Total flavonoid content

The TFC of FM biscuits extract was examined following a slightly modified method described by Mahloko et al. [37]. Biscuit sample's extract was combined with 5% $NaNO_2$ (0.3 mL) in a tube and the mixture reacted for 5 min prior to the addition of 10% $AlCl_3$ (0.6 mL). After 6 min, distilled water, and 1 M NaOH (2 mL) were added and vortexed. A spectrophotometer (UV-1600, Shimadzu, Tokyo, Japan) was employed to record the values of absorbance at 510 nm. The quercetin standard (R^2 = 0.9992) was used for standard curve and obtained results were measured in milligrams of Quercetin per gram of biscuit sample.

4.2.7.3 DPPH Radical Scavenging

The DPPH assay of FM biscuit was analysed as described by De Ancos et al. [38], wherein 3.9 mL 0.1 mM DPPH was added to the mixture of biscuit's extract (10 µl) and distilled water (90 µl). The combination was thoroughly mixed before being left in the dark to react for 30 min, after which the absorbance of the mixture was measured at 517 nm using a spectrophotometer (UV-1600, Shimadzu, Tokyo, Japan).

4.2.7.4 Ferric reducing antioxidant power (FRAP)

A method used by Lou et al. [39], was employed to measure FRAP assay of FM biscuit samples. Biscuits' sample extract (100 µL) and methanol were mixed in a test tube to make a volume of 1 mL. The content was blended with 0.2 M phosphate buffer and 1% $K_3[Fe(CN)_6]$ (2.5 mL) and mixed well and then centrifuged (Rotina 380 R- Labotech Ecotherm, South Africa) at 5000 rpm for 20 min. The obtained supernatant was combined with a mixture of 0.1 mM $FeCL_3$ (1 mL) and distilled water (1 mL). The absorbance of the combination was measured with spectrophotometer (UV-1600, Shimadzu, Tokyo, Japan) at 700 nm. A greater absorbance combination suggested a higher reducing power.

4.2.8 Physical Properties of Fermented Finger Millet Biscuits

4.2.8.1 Colour of biscuits

The top surface colour attributes (L*, a* and b*) of FM biscuits was analysed using Hunter Lab colourimeter (MiniScan XE Plus, Model CM-3500d) with a D65 light source. Before the analysis, the colourimeter was calibrated. The values for L*, a* and b* expressing the colour readings were used to calculate the chroma (C), hue angle (H°), and colour change (ΔE) using the following formulas:

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2}$$

$$\text{Hue } (H^\circ) = \tanh^{-1}\left\{\frac{b^*}{a^*}\right\}$$

$$\text{The total colour difference } (\Delta E) = \sqrt{(L - Lc)^2 + (a - ac)^2 + (b - bc)^2}$$

Where L = lightness, a = redness, b = yellowness, Lc = lightness of control sample, ac = redness of control sample, bc = yellowness of control sample.

4.2.8.2 Thickness, Diameter, Weight, and Spread Ratio

A calliper was utilised to measure the thickness and diameter of each of nine biscuit samples per formulation. A weighing balance was used to determine the weight of each of nine biscuit samples per formulation. Biscuits' spread ratio was obtained by dividing biscuit diameter by biscuit thickness.

4.2.8.3 Texture

The TA-XTplus texture analyser (stable Micro System, Surrey, UK) was used to assess the hardness of FM biscuits. A 5 kg load cell, a 3-point bend ring and a heavy-duty platform were used. The automatic settings for test speed and trigger force were 3.0 mm/s and 50 g, respectively. The peak force was recorded as hardness value of the biscuits [40].

4.2.9 Microstructural Analysis of Fermented Finger Millet Biscuits Using Scanning Electron Microscopy (SEM)

Scanning electron microscopy (Model, JSM 6610-LV, Chicago, USA) at magnification of ×1000 and scale bar of 20 µm was used to examine the morphological characteristics of FM biscuits. The samples were placed on aluminium stab with the aid of cellophane tape and coated using gold in an auto fine coater (JEO-JFC-1600) [41].

4.2.10 Sensory Evaluation of Fermented Finger Millet Biscuits

The university of Venda internal Ethics Committee granted ethical clearance (SEA/21/FST/08/1214) to the investigator before sensory evaluation was conducted. Consumer evaluation of FM biscuits amid covid-19 was conducted in the lecture hall using university consumers (staff and students) (n= 60). Panellists were asked to follow all covid-19 safety protocols before the briefing about the product and evaluation of biscuits samples for appearance, colour, aroma, taste, texture, and overall likeness using a 9-point hedonic structural scale (where 1 = dislike very much, 5 = neither like nor dislike and 9 = like extremely). The biscuit samples (8 pieces each) were presented in a white disposable plate covered with aluminium foil. Panellists were requested to taste the samples according to the plate presentation order and were required to rinse their mouths with tap water for 1 min before and after each test.

4.2.11 Statistical Analysis

The assessment of data was done by analysis of variance (ANOVA) in SPSS 26 for windows (SPSS Inc., Chicago, Illinois). The Duncan's Multiple Range Test ($p \leq 0.05$) was done to compare mean values.

4.3 Results and Discussion

4.3.1 Proximate Composition of Spontaneously Fermented Finger Millet Biscuits

Table 4.1 shows the effect of SF on the proximate composition of FM flours and biscuits. The moisture content of light and dark brown FM flours decreased significantly ($p < 0.05$) with increasing period of fermentation. When fermentation time increases, compact polymers become simpler, making it difficult to bind water resulting in the easily evaporation of water during drying [42]. Similar decrease of moisture content was observed by Adebiyi et al. [14] for spontaneously fermented pearl millet flours. There was no significant difference in the moisture content of biscuits ($p < 0.05$).

The ash content of FM and biscuits decreased as SF time increased. Higher ash content values were obtained in native FM flours and biscuits while low ash values were obtained in 72 h fermented FM flours and biscuits. The decrease in ash content of both flours and biscuits was probably due to either the leaching of mineral elements that are soluble into the fermenting medium (acid liquid) or the fermenting microorganisms' general activities which include the use of ash related components for metabolism [31]. Many studies have also reported a decrease of ash contents due to SF [14,22,43].

The fibre content of light and dark brown FM flours and biscuits showed a decrease as SF time increased.

Table 4.1. Effect of fermentation time on the proximate composition of finger millet flours and biscuits (% dry basis).

Fermentation period (h)	Moisture	Ash	Crude fibre	Protein	Crude fat	Carbohydrates	Energy (kcal/100 g)
Light brown FM flours							
0	11.56 ± 0.10^{a}	2.49 ± 0.06^{a}	2.91 ± 0.05^{a}	7.87 ± 0.42^{a}	1.82 ± 0.03^{a}	73.34 ± 0.12^{a}	347.17 ± 0.74^{a}
24	10.69 ± 0.17^{b}	1.77 ± 0.04^{b}	2.81 ± 0.02^{b}	8.29 ± 0.08^{b}	1.72 ± 0.02^{b}	74.73 ± 0.13^{b}	353.18 ± 0.79^{b}
48	10.43 ± 0.11^{c}	1.53 ± 0.03^{c}	2.71 ± 0.02^{c}	8.67 ± 0.04^{c}	1.62 ± 0.04^{c}	75.04 ± 0.08^{c}	354.85 ± 0.58^{c}
72	9.65 ± 0.15^{d}	1.10 ± 0.01^{d}	2.53 ± 0.03^{d}	9.09 ± 0.05^{d}	1.53 ± 0.02^{d}	76.10 ± 0.23^{d}	359.59 ± 0.65^{d}
Dark brown FM Flours							
0	11.78 ± 0.11^{a}	2.12 ± 0.01^{a}	3.11 ± 0.12^{a}	8.00 ± 0.04^{a}	1.75 ± 0.06^{a}	73.23 ± 0.09^{a}	346.93 ± 0.53^{a}
24	11.42 ± 0.04^{b}	1.76 ± 0.03^{b}	2.89 ± 0.04^{b}	8.41 ± 0.04^{b}	1.67 ± 0.03^{b}	73.84 ± 0.13^{b}	349.85 ± 0.19^{b}
48	10.95 ± 0.13^{c}	1.46 ± 0.10^{c}	2.74 ± 0.04^{c}	8.82 ± 0.04^{c}	1.52 ± 0.02^{c}	74.51 ± 0.08^{c}	352.49 ± 0.53^{c}
72	10.48 ± 0.06^{d}	1.38 ± 0.07^{c}	2.63 ± 0.03^{d}	9.27 ± 0.03^{d}	1.45 ± 0.02^{d}	74.79 ± 0.14^{d}	354.54 ± 0.46^{d}
Light brown FM biscuits							
0	6.91 ± 0.14^{a}	1.66 ± 0.09^{a}	3.11 ± 0.27^{a}	9.16 ± 0.37^{a}	22.11 ± 1.45^{a}	57.05 ± 0.64^{a}	470.04 ± 10.46^{b}
24	6.83 ± 0.24^{a}	1.39 ± 0.03^{c}	3.03 ± 0.23^{ab}	9.89 ± 0.57^{ab}	21.48 ± 0.86^{a}	57.38 ± 1.32^{ab}	468.46 ± 4.60^{b}
48	5.94 ± 0.67^{a}	1.24 ± 0.03^{cd}	2.89 ± 0.23^{a}	10.71 ± 0.57^{bc}	20.83 ± 1.11^{ab}	58.80 ± 1.07^{ab}	467.58 ± 4.00^{b}
72	5.93 ± 0.14^{a}	0.93 ± 0.06^{d}	2.71 ± 0.15^{a}	11.54 ± 0.71^{c}	18.83 ± 0.76^{b}	60.04 ± 0.97^{b}	461.26 ± 5.42^{a}
Dark brown FM biscuits							
0	7.60 ± 0.35^{a}	1.52 ± 0.073^{a}	3.45 ± 0.25^{a}	9.88 ± 0.78^{a}	20.75 ± 0.85^{a}	56.79 ± 2.36^{a}	460.36 ± 2.90^{b}
24	6.44 ± 0.45^{a}	1.32 ± 0.03^{b}	3.20 ± 0.09^{ab}	10.43 ± 0.47^{ab}	19.58 ± 0.49^{a}	58.03 ± 0.59^{ab}	456.94 ± 4.31^{b}
48	6.84 ± 0.89^{a}	1.25 ± 0.04^{bc}	2.96 ± 0.05^{b}	11.48 ± 0.29^{b}	18.11 ± 0.86^{b}	59.36 ± 0.32^{ab}	452.29 ± 7.72^{ab}
72	6.67 ± 2.26^{a}	1.17 ± 0.04^{c}	2.80 ± 0.10^{b}	11.66 ± 1.05^{b}	16.69 ± 1.07^{b}	61.01 ± 1.82^{b}	446.50 ± 8.18^{a}

Values are presented as ± standard deviation, n = 3. Different letters in the same column are significantly different ($p < 0.05$). LBFMB = light brown finger millet biscuits, DBFMB = dark brown finger millet biscuits.

Higher fibre content values were obtained in native FM flours and biscuits while low fibre content values were obtained in 72 h fermented FM biscuits. During SF, there are extracellular enzymes produced by microbes that are capable of hydrolysing and metabolising insoluble polysaccharide. The β-D-glucosidase hydrolyses the terminal part of polysaccharide chains which results in the decrease of crude fibre of fermented products, hence the decrease of crude fibre in the fermented biscuits [44]. Again, according to Tefere et al. [44] fermentation tends to reduce soluble fibre more than insoluble fibre, which further contributes to the reduction in crude fibre. This is congruent with report by Azeez et al. [45] who observed a decrease of crude fibre after fermentation. The carbohydrate content of light and dark brown FM flours and biscuits increased with increasing period of SF. de Olivera Silva et al. [51] observed a similar increase of carbohydrate due to SF.

The protein content of both flours and FM biscuits increased as SF time increased. The highest protein contents were obtained in 72 h fermented FM samples. A similar trend of increase of protein after fermentation has been observed by Longeria et al. [46]. The enhancement of protein content could be ascribed to the accumulation of proteins in the form of extracellular enzymes produced by lactic acid bacteria during fermentation. This is corroborated by Siezen & van Hylckama Vlieg [47], who reported the production of proteinaceous enzymes by lactic acid bacteria during fermentation, hence the increment of protein content in the fermented flours and biscuits. Other researchers have attributed the protein increment to synthesis of proteolytic enzymes during SF which hydrolyses proteins into amino acids and peptides [48,49].

The fat content of both flours and biscuits decreased as fermentation time increased. The highest fat content was found in native FM flours and biscuits, while lower fat content was found in 72 h fermented FM flours and biscuits. The fat values obtained in native biscuits were similar to those reported by Mehra and Singh [50] for biscuits prepared from pearl millet flour. The reduction of fat in the fermented samples could be attributed to lipolytic enzyme activity increase, which degrades fat components into glycerols and fatty acids through hydrolysis [22]. The increment of fat content of FM biscuits as compared to FM flours was due to the addition of fat during dough preparation. Spontaneous fermentation also increased the energy content values of light brown and dark brown FM flours and biscuits. The increment in the energy content of FM flours observed was probably due to an increase in protein contents and carbohydrates as these parameters also contribute to energy contents [22]. It could also be due to the decrease in fibre contents caused by fermentation. According Hervik [91], fermented fibre contributes to energy value by producing short-chain fatty acid (SCFA) which act as source of energy. Regarding the energy content of FM biscuits, a significant decrease in the energy value was observed in the 72 h fermented FM biscuits and this could be attributed to the fat content reduction that was observed.

4.3.2 *Mineral Compositions of Finger Millet Biscuits*

The mineral composition of fermented light and dark brown FM biscuits is presented in Table 4.2. In light and dark brown FM biscuits, the mineral content increased with the increasing period of SF. Biscuits obtained from 72 h fermented flours contained significant ($p < 0.05$) higher values of macro and micro-minerals compared to the mineral contents obtained in biscuits made from native flours. In native flour, minerals can form insoluble complexes with antinutritional factors including phytates and tannins, which can be broken during SF, resulting in the increased bioavailability of minerals [52].

Table 4.2. Influence of spontaneous fermentation on macro and micro-minerals content of finger millet biscuits. (mg/100 g dry basis).

LBFMB	Fermentation period (h)			
Macro elements	0	24	48	72
Ca	382.87 ± 1.42^{a}	386.35 ± 1.05^{b}	398.39 ± 0.41^{c}	411.33 ± 1.99^{d}
P	275.33 ± 1.23^{a}	283.41 ± 0.96^{b}	291.57 ± 0.97^{c}	299.69 ± 1.88^{d}
K	404.04 ± 1.69^{a}	410.10 ± 1.27^{b}	413.65 ± 1.06^{c}	418.50 ± 0.82^{d}
Mg	129.77 ± 1.76^{a}	134.82 ± 1.19^{b}	140.85 ± 0.81^{c}	147.94 ± 1.06^{d}
Na	7.37 ± 0.95^{a}	9.61 ± 1.16^{b}	12.25 ± 1.07^{c}	16.84 ± 1.06^{d}
Trace elements				
Cu	0.98 ± 0.15^{a}	1.86± 0.24^{b}	2.29 ± 0.14^{c}	2.74 ± 0.18^{d}
Zn	2.38 ± 0.76^{a}	3.87 ± 0.87^{b}	5.22 ± 0.80^{b}	5.91 ± 0.91^{c}
Fe	5.69 ± 0.92^{a}	7.62 ± 0.62^{b}	10.13 ± 0.67^{c}	13.22 ± 0.56^{d}
Mn	4.74 ± 0.59^{a}	7.10 ± 1.16^{b}	12.25 ± 1.07^{c}	16.84 ± 1.06^{d}
DBFMB Macro elements				
Ca	317.85 ± 1.33^{a}	335.42 ± 1.92^{b}	339.12 ± 1.49^{c}	344.88 ± 1.13^{d}
P	337.91 ± 1.84^{a}	349.92 ± 0.99^{b}	358.21 ± 1.33^{c}	364.97 ± 1.10^{d}
K	377.70 ± 1.12^{a}	388.70 ± 0.95^{b}	399.61 ± 0.59^{c}	410.01 ± 0.41^{d}
Mg	139.13 ± 1.28^{a}	148.04± 0.64^{b}	167.60 ± 0.42^{c}	174.42 ± 1.09^{d}
Na	4.86 ± 0.66^{a}	9.46 ± 0.97^{b}	14.64 ± 1.12^{c}	20.29 ± 1.01^{d}
Trace elements				
Cu	0.71 ± 0.12^{a}	1.08 ± 0.24^{b}	1.42 ± 0.12^{b}	1.79 ± 0.78^{c}
Zn	1.68 ± 0.21^{a}	2.43 ± 0.30^{b}	2.65 ± 0.12^{c}	3.39 ± 0.61^{d}
Fe	6.00 ± 0.95^{a}	7.18 ± 0.64^{a}	13.11 ± 0.79^{b}	16.03 ± 1.13^{c}
Mn	16.62 ± 1.15^{a}	21.19 ± 0.32^{b}	25.16 ± 0.70^{c}	29.20 ± 1.02^{d}

. Different superscripts in the same rows are significantly different ($p < 0.05$). LBFMB = light brown finger millet biscuits; DBFMB= dark brown finger millet biscuits.

This may explain the increase of mineral content in fermented biscuits. The mechanisms behind the liberation of minerals from phytate could be via dephosphorylation of phytate whereby phosphate groups are removed from inositol ring resulting in the decrease of phytate's mineral binding strength which enhance the bioavailability of minerals [53]. The higher improvements of minerals such as copper, iron, manganese, and zinc observed in this study could be more closely linked to SF. Chidera [54] ascribed the increase in the macro-minerals such as phosphorus to its liberation from organic complex caused by microflora enzymes. These observed increases of minerals with increasing period of SF agree with finding by Banwo et al. [55] whereby fermenting microorganisms enhanced the quality of FM and sorghum gruels when the minerals bioavailability increased.

Mineral deficiency is a shortage of dietary minerals, which are essential for good heath of humans, and it is caused by a poor diet, impaired intake of minerals after consumption or poor utilisation of minerals [56]. Calcium deficiency, iron deficiency and zinc deficiency are among the examples of mineral deficiencies that are negatively impacting the health of people. Shortage of calcium in the body has been reported to cause osteoporosis, osteomalacia, and rickets especially in Africa and Asia while insufficient supply of iron lead to anemia [57]. The deficiency of zinc causes stunting, diarrhea, and pneumonia in infants and children [57]. Therefore, the improved mineral content of fermented FM biscuits can help eradicate mineral deficiency, especially in children, if they are consumed regularly.

4.3.3 *Polyphenols and antioxidant activity of spontaneously fermented finger millet biscuits*

Table 4.3 shows the influence of SF on polyphenols and antioxidant activity of fermented FM biscuits. Unlike in previous studies [58,59], TPC and TFC in this study decreased as SF time increased. The highest TPC and TFC were found in native FM biscuits, while the lowest TPC was found in 72 h fermented FM biscuits. The decrease in TPC and TFC could be attributed to fermenting microflora's polyphenol oxidase activity [60]. It could also be attributed to abstraction of hydride ion and phenolic structure rearrangement caused by acidic environment during fermentation [61]. A similar trend of decrease in TPC after SF was observed by Adebiyi et al. [14] for GF pearl millet biscuits.

The scavenging activities (DPPH) of light and dark brown FM biscuits increased significantly with the increasing period of SF. The highest DPPH was obtained in 72 h fermented FM biscuits, while the lowest DPPH was obtained in native FM biscuits. The higher the DPPH value of biscuits, the stronger the scavenging activity of the sample. The increase in DPPH observed after SF of FM biscuits could be attributed to liberation of more soluble bioactive compounds, including oligosaccharides and peptides produced during fermentation [62]. Similar findings whereby the release of more soluble or easily extractable phenolics caused by SF which led to an increase in the DPPH in the sough dough were reported by Liukkonen et al. [63]. Srivastava et al. [64] investigated how SF affects antioxidant activity of pearl millet flour and found out that SF decreased the TPC, while increasing DPPH.

Spontaneous fermentation also influenced the iron reducing activity as significant increase was observed up to 48 h SF time in both light and dark brown FM biscuits. However, a decrease in the iron reducing activity of 72 h fermented FM biscuits as compared to other fermented FM biscuits was observed. Because TPC, TFC and antioxidant activity have negative correlation, the increase of antioxidant activity could be due to protein structural changes induced by SF. Nissen et al. [65] found that protein is involved in the enhancement of antioxidant activities by producing peptides after baking, which has stronger activity. Free sugars and protein components such as amino acids produced during fermentation boost Maillard reactions during baking, releasing additional high antioxidant-containing compounds such as melanoidin and reductones [66]. So, the possibility of an increased iron reducing activity due to conformational protein changes during SF, and baking cannot be ruled out.

Table 4.3. Polyphenols and antioxidant capacity of spontaneously fermented finger millet biscuits

Fermentation period (h)	**TPC (mg GAE/g)**	**TFC (mg QE/g)**	**DPPH (%)**	**FRAP (mg GAE/g)**
LBFMB				
0	58.78 ± 6.96^{a}	3.11 ± 0.24^{b}	52.78 ± 1.31^{a}	0.7670 ± 0.09^{a}
24	45.07 ± 4.35^{b}	3.09 ± 0.31^{b}	67.67 ± 5.30^{b}	0.9077 ± 0.03^{c}
48	37.22 ± 7.63^{b}	3.02 ± 0.05^{a}	76.42 ± 0.85^{c}	0.9480 ± 0.01^{d}
72	21.93 ± 3.56^{c}	2.97 ± 0.02^{a}	81.43 ± 1.23^{d}	0.8270 ± 0.04^{b}
DBFMB				
0	50.67 ± 0.97^{b}	3.18 ± 0.02^{c}	59.66 ± 0.94^{a}	0.8620 ± 0.06^{a}
24	49.72 ± 5.71^{b}	3.12 ± 0.03^{bc}	62.91 ± 0.47^{b}	0.8970 ± 0.04^{c}
48	33.72 ± 5.99^{a}	2.98 ± 0.05^{ab}	68.23 ± 1.13^{c}	0.9807 ± 0.01^{d}
72	29.36 ± 3.22^{a}	2.81 ± 0.17^{a}	72.92 ± 1.43^{d}	0.8800 ± 0.09^{b}

Different superscripts in the same column are significantly different ($p < 0.05$). TPC= total phenolic content, TFC = total flavonoids content.

Heat disrupts cell wall, allowing more antioxidants compounds to be released, resulting in the increase of antioxidant activity [67]. Hussain et al. [68] also found the increment of antioxidant activity of wheat-millet biscuits that was caused by some compounds produced by Maillard reaction during baking. Higher antioxidant activity obtained in fermented FM biscuits enhanced their health benefits. Antioxidants are said to act as lipid stabilizers and suppressors of excessive oxidation, which is linked to cancer and aging [69].

4.3.4 *Colour Attributes of Spontaneously Fermented Finger Millet Biscuits*

Biscuit colour as shown in Figure 4.1, is largely determined by the amount of browning that occurs during baking which is influenced by the amount of dissolved reducing sugar as well as the amount of water used [70]. Table 4 shows the colour of light and dark brown of fermented FM biscuits. The L* values of light brown FM biscuits increased significantly as SF time increased. A similar increasing trend in L* values was also found by Okin et al. [71] for biscuit made from fermented sorghum flour. As for dark brown FM biscuits, there was no significant difference in terms of the L* values.

In terms of the a* values, which represent the redness of the biscuits, there was no significant difference observed. The yellowness (b*) of light brown FM biscuits increased as

Native LBFMB (control) | 24 h fermented LBFMB | 48 h fermented LBFMB | 72 h fermented LBFMB

Native DBFMB (Control) | 24 h fermented DBFMB | 48 h fermented DBFMB | 72 h fermented DBFMB

Figure 4.1: Images of finger millet biscuits. LBFMF = light brown finger millet biscuit, DBFMB = dark brown finger millet biscuit.

Table 4.4. Influence of spontaneous fermentation on the colour attributes of finger millet biscuits.

Fermentation period (h)	L*	a*	b*	Chroma	Hue angle	ΔE
Light brown FM biscuits						
0	28.50 ± 0.81^{a}	7.89 ± 0.16^{a}	7.41 ± 0.26^{a}	10.82 ± 0.25^{a}	43.20 ± 0.88^{a}	0.00 ± 0.00^{a}
24	30.72 ± 0.64^{b}	7.82 ± 0.19^{a}	8.18 ± 0.52^{ab}	11.32 ± 0.50^{ab}	46.25 ± 1.17^{b}	2.72 ± 0.68^{b}
48	31.15 ± 0.42^{c}	8.09 ± 0.27^{a}	8.77 ± 0.49^{b}	11.93 ± 0.54^{b}	47.30 ± 0.63^{b}	2.97 ± 0.58^{b}
72	35.58 ± 0.85^{d}	8.08 ± 0.25^{a}	11.04 ± 0.77^{c}	13.68 ± 0.78^{c}	53.76 ± 1.04^{c}	7.96 ± 1.11^{c}
Dark brown FM biscuits						
0	28.87 ± 0.57^{a}	5.41 ± 0.18^{a}	6.05 ± 0.36^{a}	8.11 ± 0.34^{a}	48.15 ± 1.46^{a}	0.00 ± 0.00^{a}
24	28.99 ± 0.81^{a}	5.38 ± 0.19^{a}	6.18 ± 0.47^{a}	8.20 ± 0.48^{a}	48.88 ± 1.16^{a}	0.67 ± 0.31^{a}
48	28.54 ± 0.54^{a}	4.94 ± 0.34^{a}	5.84 ± 0.81^{a}	7.65 ± 0.82^{a}	49.58 ± 2.33^{b}	0.92 ± 0.35^{b}
72	28.70 ± 0.40^{a}	5.05 ± 0.69^{a}	5.91 ± 1.24^{a}	7.78 ± 1.39^{a}	49.26 ± 2.00^{b}	1.26 ± 0.16^{b}

Different letters in the same column are significantly different ($p < 0.05$), n = 3. L*, a*, b* and ΔE denotes lightness, redness, yellowness and total colour difference, respectively. FM = finger millet.

the SF time increased, while for dark brown FM biscuits, there was no significant difference observed. The chroma value for light brown FM biscuits increased with the increasing period of SF, while for dark brown FM biscuits, there was no significant difference observed.

Hue angle is a qualitative colour property on the basis of colours such as greenish, reddish, and yellowish [72]. Red hue is represented by a hue angle of 0° or 360°, while yellow, green, and blues are represented by angles of 90°, 180° and 270°, respectively. All the biscuits had Hue values of less than 90°, indicating that they were less yellow in the CIE-LAB colour space and had a brown spectrum in the visible region of the opponent colour chart. Regarding the total colour difference (ΔE), a lower ΔE value of 0.67 obtained in 24 h dark brown fermented biscuits indicate that SF had no effect on the colour of the biscuits, whereas higher ΔE values (1.26 & 7.96) obtained in 72 h fermented FM biscuits suggest that SF had a negative impact on the colour of the biscuits.

4.3.5 Physical properties of finger millet biscuits

Table 4.5 shows the physical characteristics of FM biscuits. The results show that SF did not have any significant effect on the diameter and thickness of the biscuits. The weight of light and dark brown biscuits decreased as SF time increased. A higher weight was obtained in biscuits made from native FM flours. High fibre content restricts more moisture loss during the process of baking which results in higher weight of the biscuits [73]. This is in congruent with Omoba et al. [74] who ascribed higher fibre content to the higher weight of biscuits. The decrease of weight in fermented biscuits could be due to decrease in fibre contents of flours (Table 4.1) during SF.

Spread ratio or diameter is determined by the flours' quality utilised in the preparation of biscuits as well as biscuits' ability to expand [75]. Spread ratio is mostly governed by the protein content of the flours [76]. The spread ratio results for light brown FM biscuits were lower in 48 and 72 h fermented biscuits. In dark brown FM biscuits, the spread ratio decreased with increasing period of fermentation. The decrease of spread ratio observed in fermented biscuits could be due to protein content increase caused by SF. Oyeyinka et al. [77] indicated that high protein content flours produce biscuits with a low spread ratio. Simply put, the higher the protein in the dough, the more water is retained, and the dough's viscosity increases, which results in a low spread ratio of the biscuits [78].

Table 4.5. Influence of spontaneous fermentation on physical properties of finger millet biscuits.

Fermentation period (h)	Diameter (cm)	Thickness (cm)	Weight g)	Spread ratio	Hardness (g)
LBFMB					
0	4.76 ± 0.05^{b}	0.66 ± 0.05^{a}	12.77 ± 0.92^{c}	7.25 ± 0.53^{c}	689.61 ± 2.70^{a}
24	4.72 ± 0.08^{ab}	0.66 ± 0.09^{a}	12.69 ± 0.41^{c}	7.25 ± 0.88^{c}	1092.07 ± 3.55^{b}
48	4.62 ± 0.08^{a}	0.70 ± 0.07^{ab}	12.53 ± 0.50^{b}	6.66 ± 0.75^{b}	1578.96 ± 1.93^{c}
72	4.68 ± 0.13^{ab}	0.76 ± 0.05^{ab}	12.10 ± 1.57^{a}	6.19 ± 0.61^{a}	2212.79 ± 3.13^{d}
DBFMB					
0	4.70 ± 0.10^{b}	0.68 ± 0.08^{a}	12.74 ± 1.09^{c}	6.98 ± 0.76^{d}	710.95 ± 2.11^{a}
24	4.64 ± 0.13^{b}	0.72 ± 0.04^{a}	12.45 ± 0.75^{b}	6.46 ± 0.29^{c}	993.20 ± 2.41^{b}
48	4.68 ± 0.13^{b}	0.76 ± 0.05^{a}	11.99 ± 0.75^{a}	6.36 ± 0.62^{b}	1474.97 ± 3.14^{c}
72	4.54 ± 0.22^{a}	0.74 ± 0.05^{a}	11.99 ± 0.84^{a}	6.05 ± 0.76^{a}	2372.23 ± 1.25^{d}

Different superscripts in the same column are significantly different ($p < 0.05$), n = 3. LBFMB = light brown finger millet biscuits; DBFMB = dark brown finger millet biscuits.

This explanation is also corroborated by Alioglu & Ozulku [79] who linked high dough protease or proteinase activity with a lower spread ratio. Similarly, Oyeyinka et al. [80] discovered that cookies made from fermented powdered cassava with a high protein content had a lower spread ratio. Texture, along with visual appearance, taste, and aroma, are important attributes of sensory quality of food [81] and their analysis and evaluation are critical during new food products development.

The hardness of light and dark brown FM biscuits increased significantly with an increasing period of SF. Basically, SF increases the hardness of biscuits and Kulthe et al. [82] ascribed it to enhanced protein-starch interaction via hydrogen bonding. It could also be due to the increase in thickness of the biscuits. Alioglu & Ozulku [80] reported that a decrease in the amount of water in the fermented dough because of the acids produced during starch hydrolysis can lead to the development of new linkages between carbohydrates, free amino acids and denatured protein which increases the hardness of the biscuits [80]. Similar hardness values were also obtained by Sulieman et al. [73] for GF baked products made from fermented *Agaricus bisporus* polysaccharide flour.

4.3.6 *Microstructural Properties of Spontaneously Fermented Finger Millet Biscuits*

Figure S1 (Supplementary Materials) shows the morphological structures of light and dark brown FM biscuits. The images of native light and dark brown FM biscuits showed a matrix of protein and lipid in which some starch granules (SG) of different sizes ranging from small to medium were embedded. Similarly, Filipčev et al. [83] described the microstructure of biscuits as a matrix of lipid and protein in which SG were embedded. Clusters of SG were also observed in the native FM biscuits. In the fermented FM biscuits (24 & 48 h), starch granules were still clearly not visible as they were still trapped in the protein lipid matrix.

However, the exposure of SG increased as fermentation period further increased. The size of the clearly visible SG varied from small to large while the shapes appeared to be spherical and polygonal. The formation of holes on SG surface were observed in the 72 h fermented biscuits as they were also observed in other fermented biscuits. This could be attributed to the decomposition of fibres (Table 4.1) by fermentation, which caused cell membrane to rapture easily when the gas expansion increased during baking, letting gas escape through the pores. The SG in 72 h fermented formed honeycomb like structure as it was also observed by Adebiyi et al. [84] for spontaneously fermented GF biscuits made from pearl millet flour.

4.3.6 *Sensory Evaluation of Spontaneously Fermented Finger Millet Biscuits*

Table 4.6 shows the organoleptic evaluation of light and dark brown FM biscuits. According to the appearance mean scores, SF did not have any significant effect on the panellist’s perception on biscuits appearance. The colour mean scores for 48 and 72 h fermented biscuits were significantly ($p < 0.05$) lower than those for 24 h and native biscuits. Panellists disliked the colour of 48 and 72 h fermented dark brown FM biscuits. This could be due to dark colour that was observed, which may have given the panellists the impression that biscuits were over-baked, influencing their preference. Aroma is the most important component that determines whether a product is accepted or not [85]. The panellists found the aroma of native and 24 h fermented FM biscuits more appreciable than that of 48 h and 72 h fermented biscuits. In terms of taste, light brown FM biscuits had higher score than dark brown FM biscuits, with native biscuits obtaining more scores than fermented biscuits. The low mean scores for taste of 72 h fermented biscuits, especially dark brown

FM biscuits could be attributable to high amounts of antioxidants and nutraceuticals and lactic acid produced during SF which might have also caused the biscuits to have unpleasant aroma [86,87].

Table 4.6. Sensory evaluation of spontaneously fermented finger millet biscuits.

	Fermentation period (h)			
LBFMB	**0**	**24**	**48**	**72**
Appearance	7.70 ± 1.56^{a}	7.67 ± 2.44^{a}	7.20 ± 2.52^{a}	7.20 ± 1.37^{a}
Colour	8.50 ± 2.20^{a}	8.32 ± 1.15^{a}	6.28 ± 1.30^{b}	5.10 ± 1.45^{c}
Aroma	7.20 ± 2.43^{a}	7.15 ± 1.22^{a}	6.25 ± 1.56^{b}	6.10 ± 1.44^{b}
Taste	7.56 ± 1.10^{a}	7.10 ± 2.46^{a}	6.70 ± 1.11^{b}	6.50 ± 2.43^{b}
Texture	6.40 ± 1.28^{a}	6.18± 1.90^{a}	6.10 ± 1.15^{a}	5.44 ± 1.17^{b}
Overall acceptability	7.80 ± 1.58^{a}	7.34 ± 2.00^{a}	7.00 ± 1.00^{ab}	6.80 ± 1.58^{b}
DBFMB				
Appearance	6.65 ± 1.44^{a}	6.50 ± 1.53^{a}	6.15 ± 1.65^{a}	6.00 ± 1.76^{a}
Colour	5.80 ± 1.34^{a}	5.50 ± 1.33^{a}	4.45 ± 1.52^{b}	4.44 ± 1.74^{b}
Aroma	6.70 ± 1.00^{a}	6.45 ± 2.40^{a}	5.98 ± 1.34^{b}	5.95 ± 1.45^{b}
Taste	7.20 ± 1.80^{a}	5.90 ± 2.00^{b}	5.40 ± 2.11^{b}	4.10 ± 2.05^{c}
Texture	6.40 ± 1.82^{a}	5.80 ± 1.10^{b}	5.56 ± 1.20^{b}	4.40 ± 1.53^{c}
Overall acceptability	6.67 ± 1.00^{a}	5.38 ± 1.58^{b}	5.35 ± 1.54^{b}	4.90 ± 1.80^{c}

Different letters in the same row are significantly different ($p < 0.05$), n = 3. LBFMF = light brown finger millet biscuits; DBFMF = dark brown finger millet biscuits.

The panellists described the aroma and taste of the fermented biscuits as bad and bitter. The findings in this study regarding aroma and taste are comparable to those of de Oliveira Silva et al. [51] and Oyeyinka et al. [78] who found low mean scores for aroma and taste of biscuits made from fermented soybean flours and cassava flours. Regarding texture and overall acceptability, light brown FM biscuits were better than dark brown FM biscuits. Biscuits made from 72 h fermented light and dark brown scored the lowest in terms of texture and overall acceptability. The low mean scores for overall acceptability of 72 h fermented biscuits could be ascribed to the bad aroma and bitter taste associated with them.

4.4 Conclusion

In this study, SF was used as a conventional and natural means to improve the nutritional composition, health promoting compounds and microstructural properties of GF FM biscuits. Spontaneous fermentation increased the protein content, carbohydrate content, mineral content, and antioxidant capacity of gluten free FM biscuits. The improved mineral content of GF FM biscuits means that daily consumption of FM biscuits can prevent mineral deficiencies in children in third world countries such as South Africa. In terms of sensory acceptance of GF FM biscuits, panelists preferred 24 h fermented biscuits compared to the rest. Spontaneous fermentation is a successful strategy to provide consumers with GF FM biscuits that are nutritious and rich in antioxidants. Further studies should be conducted on the impact of SF on amino acids, protein quality, antinutritional factors and mineral extractability of GF FM biscuits or other products that can be produced from FM flours.

Supplementary Materials: The following supporting information can be downloaded at:https://www.mdpi.com/article/10.3390/foods11091265/1, Figure S1: Scanning electron microscopy macrographs of light and dark brown finger millet biscuits; (a) Native biscuit; (b) Native dark brown FM biscuit; (c) 24 h fermented light brown fermented FM biscuit; (d) 24 h fermented dark brown FM biscuit: (e) 48 h fermented light brown FM biscuit (f) 48 h fermented dark brown FM biscuit; (f) h fermented dark brown FM biscuit; (g) 72 h fermented dark brown FM biscuit; (h) 72 fermented dark brown FM biscuit; PB= protein bodies; SG= starch granules; PLM = protein lipid matrix. FM = finger millet.

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CHAPTER 5: GENERAL CONCLUSION AND RECOMMENDATIONS.

Spontaneous fermentation (SF) has proven that it can be used in the preparation of not only bakery products but also other products such as weaning foods to address mineral deficiency in infants due to its tremendous impact on the flours' functional, thermo-pasting, and microstructural properties, as well as the mineral contents. The increased protein and mineral contents in fermented gluten-free FM biscuits suggests that frequent consumption of these biscuits may help celiac patients overcome protein and mineral deficiencies. The increment in the antioxidants activity of the fermented biscuits suggest that they can reduce the risk of hypertension, coronary heart diseases and cancer. All these shows that SF is an effective technique for providing gluten-free finger biscuits that are both healthy and antioxidant-rich to consumers. Authorities in the food industry should consider commercialising fermented gluten-free biscuits or any other food products that can made from fermented FM flours, since this may help promote the usage of FM while also enhancing the income of local rural farmers in Africa. Further studies focusing on the development of ready-to-eat food products (RTEs) from FM flours should also be prioritised. High-liquid performance chromatography (HLPC) analysis to identify and quantify polyphenols and amino acids in the fermented gluten-free biscuits should be conducted. Research focusing on the antinutritional factors and extractability of minerals as well as the microstructural analysis by X-ray diffraction (XRD) and Fourier transform-infrared (FTIR) in the FM biscuits should be done. A review study focusing on the microbial quality, biochemical properties and processes involved in the fermentation of finger millet and other cereal grain should also be done.

APPENDICES

APPENDIX A: ETHICAL CLEARANCE

ETHICS APPROVAL CERTIFICATE

University of Venda
Creating Future Leaders

RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:
Mr M Mudau

STUDENT NO:
11632442

PROJECT TITLE: **Nutritional quality, microstructural properties and consumer acceptability of biscuit obtained from fermented finger millet flour.**

ETHICAL CLEREANCE NO: SEA/21/FST/08/1214

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Dr SE Ramashia	University of Venda	Supervisor
Mr M Mashau	University of Venda	Co - Supervisor
Mr M Mudau	University of Venda	Investigator – Student

Type: **Masters Research**
Risk: **Minimal risk to humans, animals or environment (Category 2)**
Approval Period: **December 2021 – December 2022**

The Human and Clinical Trails Research Ethics Committee (HCTREC) hereby approves your project as indicated above.

General Conditions
While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The project leader (principal investigator) must report in the prescribed format to the REC:
  - Annually (or as otherwise requested) on the progress of the project, and upon completion of the project
  - Within 48hrs in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
  - Annually a number of projects may be randomly selected for an external audit.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the REC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date; a new application must be made to the REC and new approval received before or on the expiry date.
- In the interest of ethical responsibility, the REC retains the right to:
  - Request access to any information or data at any time during the course or after completion of the project.
  - To ask further questions; Seek additional information; Require further modification or monitor the conduct of your research or the informed consent process.
  - withdraw or postpone approval if:
  - Any unethical principles or practices of the project are revealed or suspected.
  - It becomes apparent that any relevant information was withheld from the REC or that information has been false or misrepresented.
  - The required annual report and reporting of adverse events was not done timely and accurately.
  - New institutional rules, national legislation or international conventions deem it necessary

ISSUED BY:
UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE
Date Considered: October 2021

Name of the HCTREC Chairperson of the Committee: Dr NS Mashau

Signature

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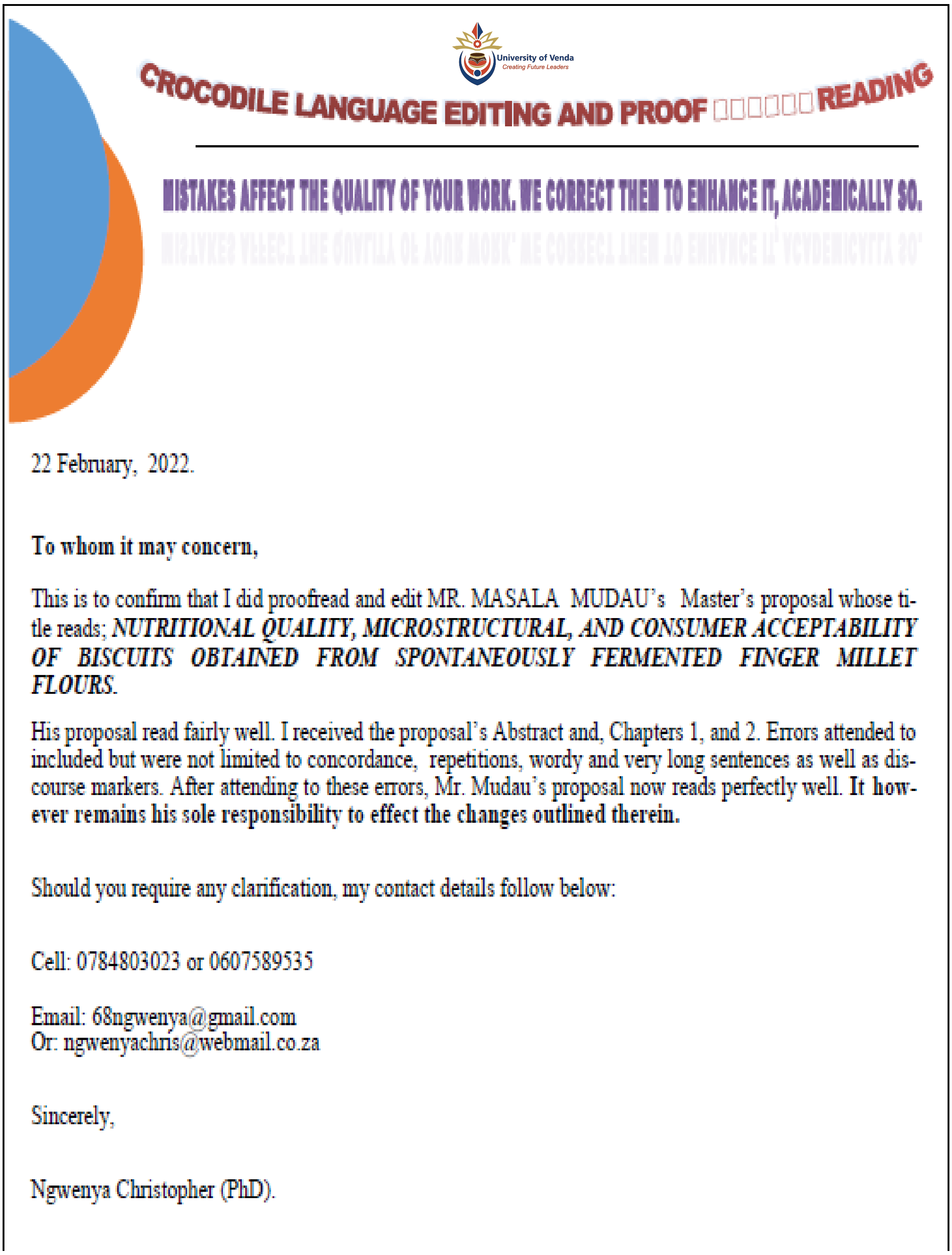
APPENDIX B: PROOFREADING REPORTS



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CROCODILE LANGUAGE EDITING AND PROOF □□□□□□ READING

MISTAKES AFFECT THE QUALITY OF YOUR WORK. WE CORRECT THEM TO ENHANCE IT, ACADEMICALLY SO.

22 February, 2022.

To whom it may concern,

This is to confirm that I did proofread and edit MR. MASALA MUDAU's Master's proposal whose title reads; ***NUTRITIONAL QUALITY, MICROSTRUCTURAL, AND CONSUMER ACCEPTABILITY OF BISCUITS OBTAINED FROM SPONTANEOUSLY FERMENTED FINGER MILLET FLOURS.***

His proposal read fairly well. I received the proposal's Abstract and, Chapters 1, and 2. Errors attended to included but were not limited to concordance, repetitions, wordy and very long sentences as well as discourse markers. After attending to these errors, Mr. Mudau's proposal now reads perfectly well. **It however remains his sole responsibility to effect the changes outlined therein.**

Should you require any clarification, my contact details follow below:

Cell: 0784803023 or 0607589535

Email: 68ngwenya@gmail.com
Or: ngwenyachris@webmail.co.za

Sincerely,

Ngwenya Christopher (PhD).

Editing and Proofreading Report

24 February 2022

This letter serves to confirm that I, Dr I. Ndlovu of the Department of English, Media Studies and Linguistics at the University of Venda, have proofread and edited an article titled "Mineral content, functional, thermo-pasting and microstructural properties of spontaneously fermented finger millet flours" by Masala Mudau, Mpho Edward Mashau and Shonisani Eugenia Ramashia.

I carefully read through the document, focusing on proofreading and editorial issues. The recommended suggestions are clearly highlighted and can either be accepted or rejected using the Microsoft Track Changes Function.

Yours Sincerely

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Editing and Proofreading Report

18 February 2022

This letter serves to confirm that I, Dr I. Ndlovu of the Department of English, Media Studies and Linguistics at the University of Venda, have proofread and edited an article titled "Nutritional quality, antioxidant, microstructural and sensory properties of spontaneously fermented gluten-free finger millet biscuits" by Masala Mudau, Mpho Edward Mashau and Shonisani Eugenia Ramashia.

I carefully read through the document, focusing on proofreading and editorial issues. The recommended suggestions are clearly highlighted and can either be accepted or rejected using the Microsoft Track Changes Function.

Yours Sincerely

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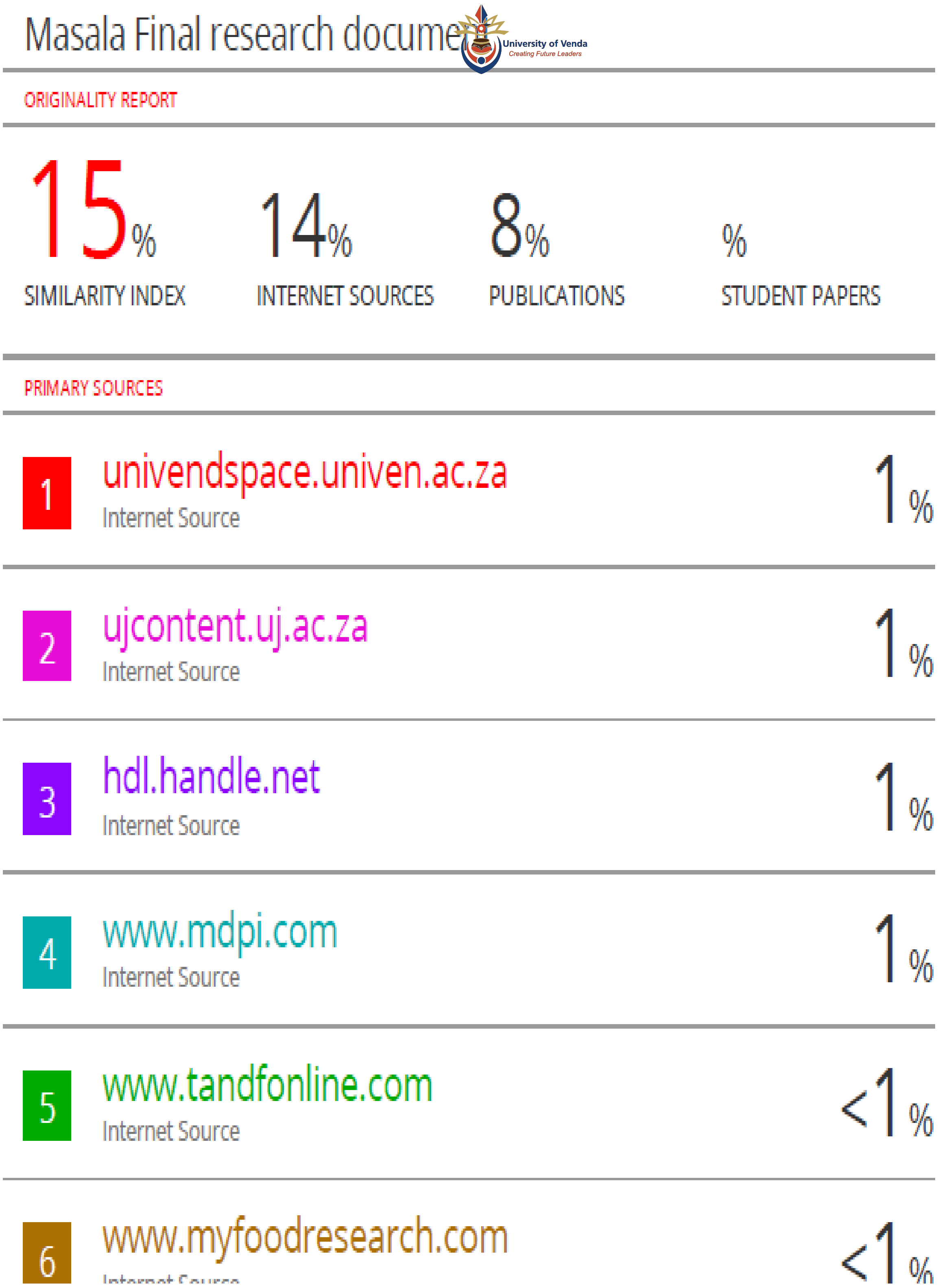
APPENDIX C: TURNITIN REPORT



Masala Final research docume

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