NUTRITIONAL, BIOCHEMICAL AND PHYSICOCHEMICAL PROPERTIES OF PEARL MILLET AND *MORINGA oleifera* COMPOSITE FOOD PRODUCTS

by

Faith Sibanda

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Supervisor: Dr Anthony O. Obilana
Co-supervisor: Professor Victoria A. Jideani

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DECLARATION

I, Faith Sibanda, declare that the content of this thesis represents my own original work, and that this thesis has not previously been submitted for academic examination toward any qualification. Furthermore, it represents my own opinion and not necessarily those of the Cape Peninsula University of Technology.

Faith Sibanda	Date
Dr Anthony O. Obilana (Supervisor)	Date
Professor Victoria A. Jideani (Co-Supervisor)	Date

ABSTRACT

Pearl millet (*Pennisetum glaucum*), contains substantial quantities of protein, minerals and vitamins and is widely cultivated in dry regions. Pearl millet (PM) flour is mostly used for making porridge or beverages for all age groups in the underdeveloped areas of Sub-Saharan Africa and Asia. To curb protein-energy malnutrition, pearl millet has been combined with legumes, to produce a more nutrient-balanced product. However, legumes present challenges such as anti-nutritional factors, poor digestibility, and toxic components. Legumes also do not address pearl millet's documented micronutrient deficiencies. This study sought to improve pearl millet's nutritional, rheological and organoleptic properties by employing malting and fermentation. Moreover, *Moringa oleifera* leaf powder (MLP) was used as a fortificant to improve the protein content and profile and the overall nutritional quality. MLP was the chosen fortificant as it is high in protein and lysine (which are deficient in pearl millet), accompanied by its exceptional overall nutrient balance. For optimum results in compositing, response surface methodology employing mixture design was applied to find optimal proportions for each of the components that yield a highly desirable protein content and or minimal saturated fat content.

Twelve mixtures with varying ratios of fermented pearl millet flour (FPMF), malted pearl millet flour (MPMF) ranging between 30–65%, and MLP ranging between 5–15% were generated through I-Optimal mixture design. The mixtures were wet-cooked, freeze-dried and analysed for protein, fat, and total phenolic content (TPC). Across the twelve mixtures, the following ranges were acquired: protein (7.3–14.2%), total fat (2.7–3.5%), saturated fat (1.3–1.6%), monounsaturated fat (0.8–1.0%), poly-unsaturated (0.8–1.0%) and TPC (129–790 $mg.g^{-1}$). The data was fitted to a linear mixture model and the search for the optimum done using Numerical Optimisation of Design-Expert (10) for maximising protein and minimising saturated fat. The linear model was suitable for explaining variation for total protein and saturated fat with R^2 of 0.50 and 0.51, respectively. Increasing MLP correlated to an increase in protein content. Two final formulations were generated through the optimisation process, (1) 15:30:55 MLP, MPMF and FPMF respectively, with 12.41% protein and 1.49% saturated fat for maximising protein with the desirability of 0.865 and (2) 15:55:30 MLP, MPMF and FPMF respectively, having 11.84% protein and 1.25% saturated fat for maximising protein and minimising saturated fat with the desirability of 0.625.

The two final formulations OS1 and OS2 were then blended, wet-cooked and freeze-dried before nutritional, biochemical and physicochemical analysis. The formulations yielded up to 22% protein and lysine content increases with ranges of 12.60–13.51 g/100 g and 0.45–0.55 g/100 g, respectively. Optimisation yielded up to a 13% reduction in saturated fat content with a resultant range of 3.89–6.59 g/100 g. Ash content was at 2.93% for both formulations translating to a 75% increase as calcium, iron and magnesium increased by over 1200%, 100% and 50%, respectively. TPC increased by up to 80% consequently effecting increases of over 25% in both oxygen radical absorbance capacity and ferric-reducing antioxidant power. Final viscosity and peak viscosity were reduced by up to 95%, respectively, a trend that translated into increased nutrient density in the cooking of gruels by reducing cooking water demand. The water solubility index increased by over 300%, while water activity decreased by up to 35%, improving reconstitutability and the keeping quality of the formulations, respectively.

Overall, fermentation increased the protein content of pearl millet whilst malting improved pasting properties such as final and peak viscosity. Mixture design and numerical optimisation were effective in determining the optimum recipe for maximising protein and or minimising saturated fat. Compositing with MLP yielded improvement in protein and mineral content, as well as phytochemical content.

Keywords: Pearl millet, *Moringa oleifera*, fermentation, malting, compositing, optimisation, mixture design

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Note: Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the Cape Peninsula University of Technology Research Funding (URF) and the In 2 Food Group (Pty) Ltd.

DEDICATION

I dedicate this project to my family, who watched and supported me grow and break bloodline perceived limits, with my first degree and kept on optimistic for a second hurdle breakthrough. To my fiancé and life partner for the late nights, lonely weekends and support activities she engaged in while I kept working on this research.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

GLOSSARY

Acronym/ Abbreviations	Definition/Explanation
AAE	Ascorbic acid equivalent
APPH	Azobis (2-methylpropionamidine) dihydrochloride
BD	Bulk density
FRAP	Ferric reducing antioxidant power
FPMF	Fermented pearl millet flour
GAE	Gallic acid equivalent
LAB	Lactic acid bacteria
MC	Moisture content
MLP	Moringa Leaf Powder
MPMF	Malted pearl millet flour
ORAC	Oxygen radical absorbance capacity
OS1	Optimisation Solution 1
OS2	Optimisation Solution 2
PM	Pearl millet
RFO	raffinose family oligosaccharides
RPMF	Raw pearl millet flour
TE	Trolox equivalent
TPC	Total polyphenol content
TTA	Titratable acidity
WAI	Water absorption index
WHC	Water holding capacity
WHO	World Health Organisation
WSI	Water solubility Index

CHAPTER ONE
MOTIVATION AND BACKGROUND OF THE STUDY

1.1 Introduction

Pearl millet *(Pennisetum glaucum)* is widely cultivated in arid and semi-arid regions in India and areas that are characterised by low rainfall and infertile soils, on which other major cereals fail to yield significant harvests (Manga & Kumar, 2011:200). In South Africa, pearl millet is cultivated in the Free State, Limpopo, and KwaZulu-Natal provinces. Pearl millet flour is mostly used to make porridge or beverages, for large populations mainly in the underdeveloped areas of Sub-Saharan Africa, India and China. It provides protein with a substantial quantity of essential amino acids as well as fatty acids, minerals, vitamins and polyphenols (Issoufou *et al*., 2013:501). However, pearl millet presents a deficiency of lysine, an essential amino acid, which is often compensated by compositing with protein-rich legumes.

In efforts to curb protein-energy malnutrition and supplement other nutritional shortfalls, pearl millet has been combined with legumes, a process known as compositing. Compositing or blending involves combining two different nutrient sources, to produce a more nutrient-balanced meal. Legumes comprising cowpea, chickpea, pigeon pea, soybean and pulses have been used to improve protein content in cereals (Nnam, 2001:251; Anyango, 2009:1; Pelembe, Erasmus, *et al*., 2002a:120). However, they also present challenges such as the introduction of antinutritional factors, poor digestibility, and raffinose family oligosaccharides (RFOs) that cause flatulence (Gebrelibanos *et al*., 2013:1271). Moreover, there is a need to control ‘hidden hunger’, a term that describes micronutrient deficiencies, that is prevalent in Eastern and Southern Africa where maize is a staple food across all age groups (Galani *et al*., 2022:1568).

Malting and fermentation or enzymatic treatment have been suggested to improve the nutritional, biochemical, physicochemical, and functional properties (due to protein and carbohydrate structural modifications) of pearl millet (Issoufou *et al*., 2013:504). These processing methods assist in producing lower-viscosity pearl millet food products with reduced content of antinutritional factors and improved nutrient bioavailability. Such pearl millet food products are benefitting to a different consumers, which include weaning-stage infants, patients or even anyone (for example athletes) in need of semi-liquid or low-viscosity meals.

Moringa oleifera, a tree legume cultivated in the tropics and subtropics, often called the ‘miracle tree’, is highly valued in the medical and food sector. Moringa leaf powder (MLP) presents a great opportunity for improving the nutritional quality of

pearl millet-based foods as it is highly nutrient-dense with an exceptional overall nutrient balance as found by (Moyo *et al*., 2011:12925; El-Fatah *et al*., 2013:1065; Okereke & Akaninwor, 2013:34). *Moringa oleifera* leaves are reportedly a source of lysine (deficient in pearl millet) and other essential amino acids, presenting substantial amounts of vitamin C and minerals notably iron, zinc, and calcium. Moringa leaf poweder also offers substantial phytochemicals to impart nutraceutical functionalities in pearl millet foods and renders it a suitable alternative to legumes for compositing with cereal grain flours.

Mixture experiments are response surface experiments used in designing products whereby the product under investigation is made up of several components. Statistical analysis and mixture design are utilised in synergy to evaluate the effect of combining two or more components and to forecast the response(s) for all probable formulations of the mixture. In this study, mixture design is used to find optimal proportions for each of the components (malted pearl millet and fermented pearl millet flour and moringa leaf powder) to be chosen to yield a highly desirable response for protein content and or minimal fat content (Giese *et al*., 2011:239; Goos *et al*., 2016:899; Wangkananon *et al*., 2018:176).

1.2 Statement of the Problem

Pearl millet is one of the staple grains in the arid and semi-arid regions of Africa and India. However, it is deficient in selected amino acids, especially the essential amino acid, lysine, and also has poor digestibility. Cooking pearl millet gruel often requires large quantities of water to reach good paste consistency, which leads to a lower nutrient density of the cooked food (Donnen *et al*., 1996:146). Legume composites have been investigated and documented to significantly improve the protein content of cereals. However, most studies yielded low protein digestibility while some indicated the disadvantage of the introduction of flatulence-causing raffinose family oligosaccharides (RFOs), amongst other antinutritional factors (Gebrelibanos *et al*., 2013:1271). Moreover, compositing legumes with cereals has not adequately addressed the micronutrient deficiencies in pearl millet and other cereals. Compositing pearl millet flour with *Moringa oleifera* leaf powder (MLP) and employing malting and fermentation processes in the processing of pearl millet, appear to be a solution in providing the complementary amino acids and other micronutrients with the added advantages of better overall nutrient density and nutrient balance (Yang *et al*., 2006:2; El-Fatah *et al*., 2013:1065; Okereke & Akaninwor, 2013:34)

1.3 Objectives of the research

The main objective of this research project is to develop a nutrient-dense composite flour consisting of pearl millet with *Moringa oleifera* leaf powder.

The specific objectives are to:

1. Determine an optimum compositing ratio(s) of malted pearl millet flour, fermented pearl millet flour and MLP to attain a maximum protein content and or minimum saturated fat content.
2. Establish the impact of malting and fermentation on the nutritional, biochemical and physicochemical properties of pearl millet.
3. Produce fermented, malted pearl millet and moringa leaf composite instant flours based on mixture design and optimisation outcomes.
4. Assess the impact of compositing pearl millet with MLP on the nutritional, biochemical and physicochemical properties of the composite food products.

1.4 Hypothesis

In this study, it is hypothesised that:

1. Compositing pearl millet with MLP will significantly improve the protein content of pearl millet food products (El-Fatah *et al*., 2013:1068).
2. Compositing pearl millet with MLP will improve the mineral content and nutrient density (El-Fatah *et al*., 2013:1069).
3. The application of malting and fermentation will improve the pasting properties of the pearl millet and MLP composite (Ikujenlola, 2008:471).

1.5 Delineation of the research

The study utilised only one variety of pearl millet (Babala) and *Moringa oleifera* leaf powder.

1.6 Significance of Research

The study seeks to improve the nutrient quality of pearl millet in a bid to overcome undernutrition especially, protein-energy malnutrition associated with poor starchy cereals. Compositing with moringa may not only solve the pearl millet lysine deficiency but also improve the vitamin and mineral profile of meals and prove to be an efficient yet affordable means of fortification to tackle micronutrient deficiency that is prevalent in Eastern and Southern Africa (Galani *et al*., 2022:1568). Moreover, there are prospects of producing a market-viable product that does not only serve as a nutrient source but also as a phytochemical-rich nutraceutical food.

1.7 Outcomes and Results of this Study

An optimum compositing ratio of malted pearl millet and fermented pearl millet flours and MLP for maximum protein and or minimum fat content will be established. The protein content, amino acid profile and mineral content of pearl millet foods will be improved in the final product. Fermented, malted pearl millet and moringa leaf composite instant flours readily reconstituted into a beverage or porridge will be produced. At least one research article will be published in a DHET-accredited journal and one local or international conference will be attended for the presentation of the research output. A Master of Food Science and Technology degree is expected to be awarded from this study.

1.8 Thesis Overview

This thesis contains five chapters and was written in a journal article format where each chapter looks like a manuscript.

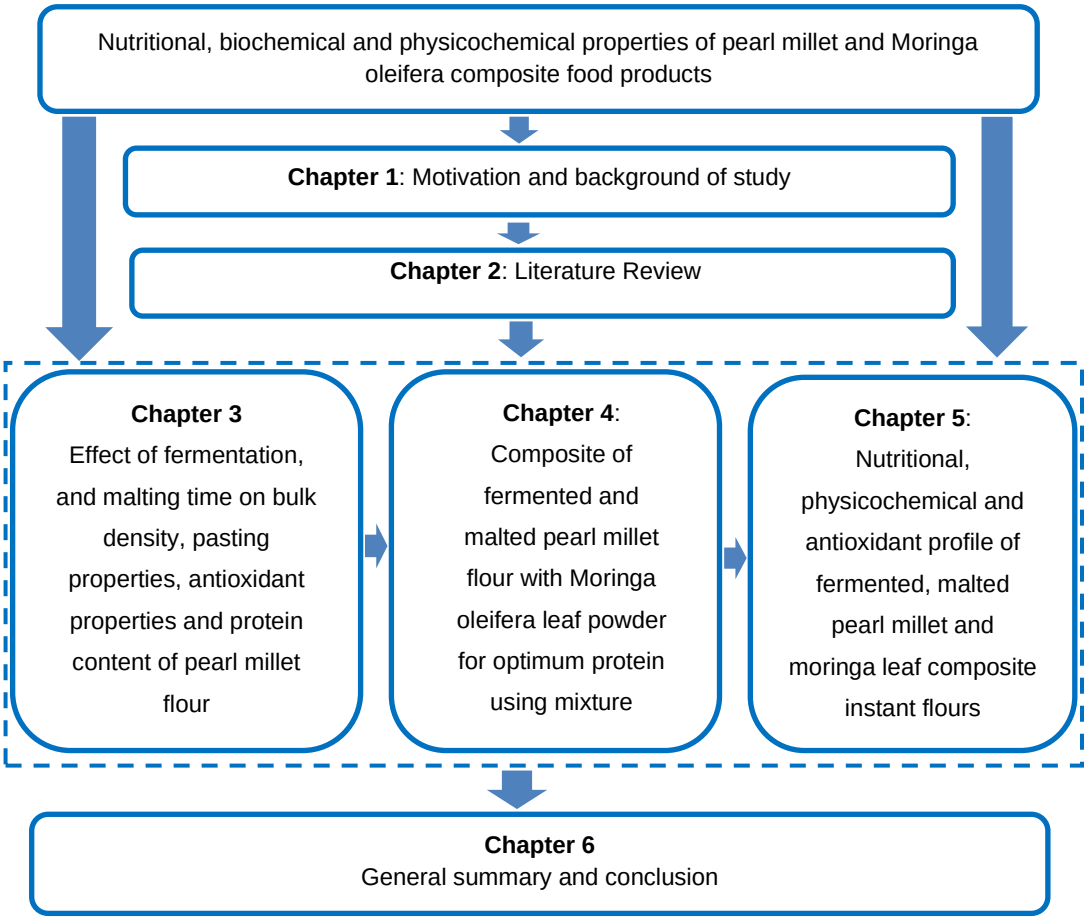

Figure 1.1: Thesis Overview.

Chapter one is the introduction which details the motivation, design and background of the research and highlights the statement of the research problem as well as the objectives of the study. Moreover, the research theory, delineation and significance of the study and the expected outcomes are detailed in the chapter. Chapter two entails a literature review providing a more detailed outline of the research background in terms of raw material taxonomy and composition, processing methods and their impact thereof. Chapter three, the first chapter on the research findings, outlines the processing of raw pearl millet, that is malting and fermentation. The chapter further details the effect of both malting and fermentation times on pasting properties, protein content, total phenolic content and antioxidant properties of pearl millet flour. Chapter four describes experimental design starting with the application of I-optimal point exchange mixture design and the composition of twelve mixtures generated thereof. The chapter goes on to highlight analysis, that is, nutritional content (protein and fat) and total phenolic content conducted on the twelve mixtures for the optimisation process. The last part of the chapter dwells on evaluating model fit and adequacy as well as the effect of the mixture components on applicable responses before concluding with solutions from the numerical optimisation process. Chapter five discusses the production of two fermented, malted pearl millet and moringa leaf composite instant flours acquired from Chapter four (numerical optimisation) and delves into the nutritional, biochemical and physicochemical analysis of the composite flours. The major focus of this chapter is on the impact of augmenting pearl millet flour with MLP to improve the protein and amino acid content, as well as the micronutrient profile. Chapter six is the concluding chapter as it entails a summary of the research findings, the conclusions reached and recommendations for future research.

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CHAPTER TWO LITERATURE REVIEW

The following review of literature encompasses the overview of pearl millet as a crop, its nutritional quality and general processing techniques, and their impact on nutritional, biochemical and physicochemical properties. The chapter further looks at food compositing as a method of augmenting the nutritional quality of pearl millet, with a focus on *Moringa oleifera* leaf powder as an alternative to conventional legume options. Response surface experiments are also briefly covered as scientific and objective methods of determining the mixture for achieving desired optimum results.

2.1 Pearl Millet

2.1.1 Taxonomy, Climatic Requirements and Morphology

The millet family is sometimes classified in the Paniceae family with other sources suggesting Gramineae (Mckevith, 2004:112). Falling under the genera Panaceae, pearl millet (*Pennisetum glaucum*), which is consumed either as porridge or beverage, is one of the major sources of protein and energy for millions of people in the underdeveloped regions of Sub-Saharan Africa, India and China (Issoufou *et al*., 2013:501). In South Africa, pearl millet is cultivated in the Free State, Limpopo, and KwaZulu-Natal provinces. Pearl millet's drought tolerance and resistance to insect damage during storage give it a superior preference over most other grains in arid or famine-susceptible regions with rainfall as low as 150 mm per annum and temperatures up to 43°C (Yadav *et al*., 2016:209).

Similar to sorghum, the pearl millet caryopsis is found in various shapes (tear-shaped to ovoid) among existing varieties with sizes up to 2 mm long and 2.25 mm wide, and colours such as yellow, grey and pearly white (Rooney & McDonough, 1987:43; Kajuna, 2001:2; Bryden *et al*., 2013:3). The principal parts of the pearl millet grain are the endosperm, germ and pericarp, which make up 73.9 to 76.2% (82.3% in sorghum), 15.5 to 17.4% (9.8% in sorghum) and 7.2 to 10.6%, respectively (Bryden *et al*., 2013:3; Abdelrahman *et al*., 1984:127). This architecture is said to impact nutrient composition indicated by higher fat and protein contents of pearl millet compared to sorghum (Hoseney *et al*., 1987:398; Sullivan *et al*., 1990:84; Bryden *et al*., 2013:3).

2.1.2 Nutritional Quality

Table 2.1 shows the macronutrient and amino acid content of pearl millet as compared to wheat, sorghum, and standard World Health Organisation (WHO) requirements.

Table 2.1 Macronutrient and amino acid composition of pearl millet compared to other grains and standard requirements

Component	Pearl millet[a,b]	Sorghum[a,c]	Wheat[a,d]	WHO Standard[e]
Macronutrients (g)				
Protein	11.8	8.4	11.8	-
Fat	4.8	3.3	1.5	-
Crude fibre	2.3	1.6[a]	1.2	-
Carbohydrates	67	76	71.2	-
Essential amino acids (g /100 g protein)				
Leucine	14.1	12.8	6.8	5.9
Isoleucine	5.1	3.7	3.7	3.0
Lysine	0.5	2.1	2.8	4.5
Methionine	1.0	1.7[1]	1.2[1]	2.2[1]
Phenylalanine	7.6	5.2[2]	6.4[2]	3.8[2]
Threonine	3.3	3.7	2.9	2.3
Valine	4.2	4.6	4.4	3.9
Histidine	1.7	2.0	2.3	1.5
Tryptophan	1.2	1.3	1.1	0.6

1. Methionine + cysteine

2. Phenylalanine + Tyrosine

a. Adapted from Nambiar *et al*. (2011:63)

b. Adapted from Issoufou *et al*. (2013:502)

c. Adapted from USDA (2015)

d. Adapted from Shewry (2009:1542)

e. Requirements patterns WHO (2007)

Pearl millet protein is gluten-free, rendering it suitable for sufferers of celiac disease, a digestive autoimmune disorder of gluten (Nambiar *et al*., 2011:63). The protein content (11.8 g/100 g), in Table 2.1, is more than that of sorghum (8.4 g/100 g) but similar to wheat (11.8 g/100 g) with an amino acid balance comparable to sorghum. However, like many other cereal grains, pearl millet lacks lysine, threonine, tryptophan, and other sulphur-containing amino acids such as methionine and cysteine.

Pearl millet generally has a higher fat content (3 to 6%) when compared to other grains and this has been linked to its generally poor keeping quality. Unsaturated fatty acids comprise 75% of the total fat, with omega-3 linoleic acid comprising 4% of the total fatty acids. Essential fatty acids that are present include alpha-linolenic acid, docosahexaenoic acid and eicosapentaenoic acid, which are all polyunsaturated fats

and also omega-3 fatty acids. The carbohydrate content appears to be the lowest (67%) comprising between 17.0 to 21.5% amylose and 78.9 to 83.0% amylopectin (Nambiar *et al*., 2011:64).

Table 2.2 shows the mineral and vitamin content of pearl millet as compared to wheat and sorghum.

Table 2.2 Mineral and vitamin content of PM compared with other cereals per 100 g

Constituent	Pearl millet	Wheat	Sorghum
Minerals (mg)			
Calcium	42	41	1.6
Phosphorus	296	306	222
Iron	11	5.3	4.1
Zinc	2.2	2.7	1.6
Sodium	10.9	17.1	7.3
Magnesium	137	138	171
Vitamins (mg)			
Vitamin A*	132	64	47
Thiamine	0.38	0.45	0.37
Riboflavin	0.21	0.17	0.13
Niacin	2.8	5.5	3.1
Folic Acid*	45.5	36.6	20

*Values reported in µg

Adapted from Nambiar *et al*. (2011:63)

The total mineral content of pearl millet is about 2.2 g/100 g comprising predominantly calcium, zinc, iron and magnesium. The vitamin and mineral contents of pearl millet are higher than those of sorghum which only have slightly higher values for niacin and Mg, 5.5 mg/100g and 171 mg/100 g when compared to 2.8 mg/100 g and 137 mg/100 g of pearl millet, respectively. The mineral content of pearl millet is comparable to the mineral content of wheat, with slightly higher Ca and notably superior Fe content.

The pericarp of pearl millet, which makes up about 8.4% portion of the total grain, contains substantial quantities of nutrients, protein (9.4%), fat (5.9%) and ash (13.9%) of the total respective nutrients in the grain (Bryden *et al*., 2013:3). The present study intends to incorporate the substantial nutrients in pericarp by utilising whole pearl millet grain by excluding the decortication of the pearl millet grains.

The overall protein digestibility of pearl millet is determined by the ratios of protein fractions comprising albumins, globulins, true and pseudo prolamins and true and pseudo glutelins. A comparative study by Ejeta & Hassen (1987:6017) concluded that pearl millet has a superior *in vitro* pepsin digestibility to sorghum in a study of two pairs of sorghum and pearl millet varieties where two varieties yielded 89% and 91% whilst sorghum hybrids yielded 82% and 89%. Contrary to these findings Pushparaj & Urooj (2014: 898)reported a low pearl millet protein digestibility range of between 45–49%. The digestion of pearl millet amylose by β-amylases to maltose (amylolysis) was found to be slower (73.9% β-amylolysis limit) when compared to wheat (67.2% β-amylolysis limit), due to the presence of more orderly arranged molecules of pearl millet starch, branching in its amylose and the presence of amylolytic inhibiting polyphenols (Nambiar *et al*., 2011:63; Nambiar *et al*., 2012:256).

The bioavailability of major minerals such as Ca, P, Zn, Fe, Cu and Mn are affected by the phytic acid content as it binds these minerals in complexes (Eltayeb *et al*., 2007a:463; Coulibaly *et al*., 2011:1). According to Kumar & Chauhan (1993:505), the HCl extractability of minerals Ca, Fe, Zn and P were 32.4%, 27.6%, 46.8% and 39.8% respectively whereas Khetarpaul & Chauhan (1991:352) reported 36.0%, 19.4%, 42.2%, 35.2% and 52.8% HCl extractability for Ca, Fe, Zn, Cu and Mn, respectively in pearl millet.

Phenols which have been found in substantial quantities in millets are characterised by the presence of an aromatic ring with one or more hydroxyl rings (Shahidi & Naczk, 2004:36). Some of the phenolic compounds are potentially chemo-preventive and have been suggested to possibly prevent cardiovascular and neurodegenerative diseases associated with oxidative stress (Issoufou *et al*., 2013:504). A study of seven pearl millet varieties reported a range between 268.5 to 420 mg/100 g of total phenols, comprising dietary antioxidants flavonoids and phenolic acids (Nambiar *et al*., 2012:255). However, some phenolic compounds such as phytates and phytic acid, polyphenols, tannins and goitrogens can also be antinutritional (Bryden *et al*., 2013:13).

The most prevalent antinutritional phenolic, phytic acid or phytate, is a phosphorus-containing compound named myo-inositol-1,2,3,4,5,6-hexakisphosphate. Its antinutritional element, which is exhibited by its phytic acid form, is the ability to readily form complexes (chelating) with multi-valent metal cations (Ca, Fe, Mg and Zn) and proteins limiting their bioavailability (Bryden *et al*., 2007:279; Bryden *et al*., 2013:13). It is found in the germ and aleurone layers of pearl millet in the range of 1.7 to 3.3 g/kg (Taylor, 2004:255; Bryden *et al*., 2013:14).

2.1.3 General Processing Techniques for Pearl Millet

Traditionally, the processing of pearl millet involves cleaning, soaking, germination and drying followed by fermentation depending on the desired product (Issoufou *et al*., 2013:505). Commercial methods of pearl millet processing comprise dehulling, soaking, blanching, autoclaving, germination, roasting and fermentation (Coulibaly *et al*., 2011:6; Legesse, 2013:21; Bryden *et al*., 2013:15).

2.1.3.1 Malting

Malting is a triple-step procedure comprising steeping (soaking), germination (of cereal grains in a moist, controlled atmosphere to aid the accumulation of hydrolytic enzymes which are otherwise absent in raw (unprocessed) grains and drying (kilning). Drying serves two purposes namely flavour and aroma development and growth termination which takes place once enzymes needed for the degradation of starch and proteins have been produced in substantial amounts (Lyumugabe *et al*., 2012:513).

Soaking or steeping, involves soaking the grain in water to initiate germination and create desired changes in the endosperm for the malt production process (Lyumugabe *et al*., 2010:4241; Lyumugabe *et al*., 2012:510). The process can take up to 48 h, depending on the grain type, desired malt quality and temperature, with sufficient aeration to preserve the embryo. Steeping temperatures vary from 10 to 16°C for longer steeping times of up to 48 h and 20 to 35°C for 8 h wet (static water) and air-rest cycles (Pelembe, Dewar *et al*., 2002:7; Lyumugabe et al., 2010:4241). Taylor (2008:9) recommended 0.3% caustic soda (sodium hydroxide) at temperatures of between 22–28°C for up to 6 h with periodic agitation. Key parameters in the absorption of moisture into the grains during steeping comprise time and temperature with steep moisture of between 42–48% regarded to be optimal for enzyme activity (Pelembe, Dewar *et al*., 2002:7; Lyumugabe *et al*., 2010:4241; Lyumugabe *et al*., 2012:510). Soaking reportedly reduced phytic acid by 16 to 21% and with another study indicating a 39.5% reduction during a 24 h and 18 h period of soaking grains (Mahgoub & Elhag, 1998:77; Eltayeb *et al*., 2007b:464). Up to 43.9% reduction of total phenols was noted during the soaking of three varieties of sorghum and this was linked to the leaching of phenols into the soaking medium (Afify *et al*., 2012:205).

Germination is conducted by spreading out steeped grains to form a layer of about 2–3 cm in thickness where grains are then covered with clean plastic sacking or plastic shade cloth and left at ambient temperature (25°C) for between 2–5 days (Taylor, 2008:10). The layers must be turned over at least twice a day with periodic water sprinkling to maintain the initial moisture level (Lyumugabe *et al*., 2010:4241; Lyumugabe *et al*., 2012:511). The germination process encompasses the outgrowth of

the plumule and radicle of the seedling, a process characterised by the development of starch-degrading enzymes and proteases (Palmer, 1989:313).

Inyang & Zakari (2008b:11) reported an almost 18% protein content increase in germinated pearl millet flour. Germination was also reported to increase *in vitro* protein digestibility of pearl millet by up to 42.7% and this was attributed to an increase in soluble proteins due to the action of endogenous enzymes partially hydrolysing storage proteins making them better suited for pepsin digestion (Pushparaj & Urooj, 2011:898). Sripriya *et al.* (1997:348) and Khetarpaul & Chauhan (1990a:24) reported a 12% starch content decrease that was attributed to mobilisation and hydrolysis by endogenous amylases. Pearl millet total soluble sugars increased from 1.76 g to 6.13 g/100 g while reducing sugars increased from 0.36 g to 3.43 g/100 g due to starch hydrolysis (Khetarpaul & Chauhan, 1990a:23).

Kumar & Chauhan (1993:73) reported increases of up to 100% and over in pearl millet HCl extractability of Ca and Fe while Zn increased by up to 40%. This was accompanied by phytic acid content reduction which Nkhata *et al.* (2018:2448) pointed out to be a result of phytic acid hydrolysis by endogenous phytase. Kumar & Chauhan (1993:72) reported a 91% reduction in phytic acid which was higher than the 44.7% reduction that was reported by Inyang & Zakari (2008b:11) after germination at temperatures between 25 to 35°C for up to 48 h of germination, probably due to variations in reaction kinetics (pH, time and temperature).

On physicochemical and functional aspects, germination significantly ($p < 0.05$) increased water absorption capacity by up to 7.8% in sorghum, an attribute reported to reduce the problem of dietary bulk common in starchy foods (Nnam, 2001:241; Elkhalifa & Bernhardt, 2010:390). Starch hydrolysis to simple sugars reportedly reduces the swelling capacities of cereal flours during cooking, consequently improving gruel consistency (Ikujenlola, 2008:473). Sprouted wheat samples exhibited much lower peak viscosity and hot paste viscosity than non-sprouted samples, an indication that the water binding capacity and starch paste stability had been decreased by sprouting (Borijindakul & Phimolsiripol, 2013:69; Simsek *et al*., 2014:194). Simsek *et al.* (2014:200) attributed this occurrence to an increase in α-amylase activity which affects the branch chain length of amylopectin and consequently impacts the gelatinisation, retrogradation and pasting properties of starch.

Germinated grains are then dried or kilned, exposing them to slightly elevated temperatures until the chits or rootlets become brittle. The key goals are terminating embryo growth and enzyme activity while minimising enzyme denaturation, simultaneously aiding the development of flavour and colour (melanoidin compounds). The process can be carried out in a kiln or oven at varying temperatures, with 50°C for

up to 48 hours mainly recommended. A two-stage kilning technique (first stage at 55°C and subsequent stage at 65°C) has been recommended to produce good quality malts with notable moisture reduction and sugar content as well as malt flavour development while minimising the destruction of hydrolytic enzymes (Owuama & Asheno, 1994:257; Lyumugabe *et al*., 2012:513). A study of two pearl millet varieties indicated that roasting notably improved *in vitro* protein digestibility for 2 varieties of pearl millet by 20.3% and 26.1%. The improvement brought by heat treatment was attributed to protein denaturation (Pushparaj & Urooj, 2011:898).

2.1.3.2 Decortication and Milling

Decortication commonly referred to as dehulling (as derived from grains with hulls) is the removal of the pericarp layers from the cereal grain to improve the texture, colour and cooking quality of the grain (Scheuring & Rooney, 1979:545; Barrion, 2008:83). Traditionally, the decortication of millets is carried out by first mixing the grains with water and briefly soaking and pounding them using a mortar and pestle with subsequent drying and winnowing to eliminate bran and other fine extraneous material. Modern dehulling involves the use of mechanical dehullers, attrition and abrasion equipment, to remove the pericarp layers from the grain (Reichert & Youngs, 1977:176).

Decortication is reported to reduce phytic acid and total polyphenols by up to 49.8% and 22.4% respectively. This correlated with an increase *in vitro* protein digestibility (8.8 to 11.6%) (El Hag *et al*., 2002:193). The trend indicates positive dehulling effects in grain flour quality as phytic acid reportedly lowers protein digestibility and mineral extractability (Coulibaly *et al*., 2011:4). However, the removal of the pericarp which often comes off with part of the endosperm results in the reduction of nutrients such as protein, oils and minerals that are contained in these layers of the grain (Serna-Saldivar & Rooney, 1995:70; Kebakile, 2008:5; Bryden *et al*., 2013:3). For example, El Hag *et al.* (2002:194) reported a 12.6% protein loss during dehulling of pearl millet varieties. Bryden *et al.* (2013:3) stipulated that the pericarp contains 13.9% of the total ash in the pearl millet grain; hence its removal translates into mineral losses of almost that magnitude. Moreover, Nambiar *et al.* (2011:64) stated a 50% reduction in thiamine, riboflavin, and niacin content as a result of decortication.

Milling, a process that is sometimes incorporated with dehulling, involves the conversion of seeds into flour (Lorenz & Dilsaver, 1980:16). Taylor (2008:6) recommended hammer and disk (attrition) mills for sorghum and millet malt flours. In traditional milling, Rooney & McDonough (1987:44) reported that a wooden mortar and

pestle are used to thresh, decorticate and grind grain into flour or meal with slight water addition to moisten the pericarp, and facilitate bran removal.

2.1.3.3 Fermentation

Fermentation is the energy-generating metabolic process by which carbohydrates are oxidised in the absence of an external electron acceptor due to the action of microorganisms and enzymes (Coulibaly *et al*., 2011:8). Conventional fermentation is carried out in a slurry made with about a 1:2 ratio of flour to water. A homogeneous mixture is achieved by first adding about 50% of aliquots (usually water) with the gradual subsequent addition of other remaining portions and the inoculum, where applicable. The slurry is briefly pre-cooked and cooled to incubation temperatures (25 to 35°C) for between 24 and 72 h. In spontaneous fermentation, the process is started without the inoculum and back-slopping then becomes the subsequent inoculation technique between batches whereby part of the fermented mass is added to the next batch as inoculation medium (Sahlin, 1999:12). Fermentation can be performed by the action of natural Lactic acid bacteria (LAB) or by inoculation with cultures of LAB species namely *Lactobacillus plantarum*, and *Lactobacillus fermentum* (Sahlin, 1999:32).

Fermentation improves protein quality as well as lysine, methionine and tryptophan levels in millet (Nanson & Fields, 1984:959; Hamad & Fields, 1979:456). A study conducted on two pearl millet cultivars revealed that fermentation resulted in up to a 15.9% increase in the *in vitro* protein digestibility of standard and Ugandi cultivars of pearl millet after 14 h of fermentation (El Hag *et al*., 2002:195). Natural fermentation has been suggested to decrease carbohydrates and some non-digestible poly- and oligosaccharides due to the hydrolysis of starch and processing of fermentable sugars to produce organic acids such as lactic acid, acetic acid and pyruvic acid (Mugula *et al*., 2003:187; Coulibaly *et al*., 2011:8). Alka *et al.* (2012:69) reported an *in vitro* starch digestibility increase of 49% in the fermentation of pearl millet and attributed this to changes in the endosperm protein fractions availing starch to digestive enzymes.

Fermentation causes a drop in pH leading to optimum conditions (pH = 5.5) for enzymatic degradation of polyvalent phytic acid complexes to liberate bound Zn, Fe, Ca and Mg cations (Coulibaly *et al*., 2011:8). Endogenous phytase hydrolyses phytic acid into lower myo-inositol phosphate esters with a lower ability to bind multivalent mineral cations (Leenhardt *et al*., 2005:98). A study carried out on the effects of germination and fermentation on instant *fura* (thick cereal porridge) processing, showed that the phytic acid level was reduced by 36% in fermented *fura* (Inyang & Zakari, 2008b:11). El Hag *et al.* (2002:195) reported up to 60% total polyphenol

reduction after 14 h of traditional pearl millet fermentation, while (Osman, 2011a:4) reported up to 51.9 % phytic acid reduction after 24 h of sorghum fermentation.

The reduction in total phenolic content achieved during pearl millet fermentation is reported to increase the mineral extractability of Fe, Zn, Ca and Mg (Sripriya *et al*., 1997:345; Coulibaly *et al*., 2011:9; Nambiar *et al*., 2012:256). According to Khetarpaul & Chauhan (1990b:350), natural fermentation of pearl millet results in an 84, 45 and 47% increase in HCl extractability of Ca, Zn and Mn, respectively, while over 100% increases were observed for Fe and Cu. These findings support assertions by Coulibaly *et al.* (2011:8) and Leenhardt *et al.* (2005:98) who attributed the increase in mineral extractability to phytic acid degradation that in turn liberates bound minerals availing them of more efficient extraction.

Fermentation also affects the organoleptic and keeping qualities of pearl millet flour as numerous chemical compounds produced improve the shelf life, texture, taste and flavour of the finished product. The presence of diacetyl acetate and butyric acid contributes greatly to the overall flavour (Blandino *et al*., 2003:529). The drop in pH during fermentation imparts antimicrobial properties (acid environment) enhancing the keeping quality and minimising the possibility of food poisoning of millet flour and its food products (Inyang & Zakari, 2008b:11; Giese, 1994:102).

Improvements in various physicochemical and functional properties have also been reported because of fermentation, as endogenous amylases and proteases hydrolyse starch and peptide linkages between amino acids, hence destabilising complex starch molecules and firm protein networks, respectively, consequently decreasing the viscosity of the paste (Hallén *et al*., 2004:181). A decrease of about 8.5% in water absorption capacity was reported after 36 h of pearl millet fermentation, a trend affirmed by Elkhalifa *et al*. (2005:4) in sorghum fermentation, hence imparting an attribute known to improve textural properties (paste viscosity and consistency) of gruels (Ikujenlola, 2008:471; Alka *et al*., 2012:68). A period of 36 h of fermentation resulted in a 16.9% reduction in the bulk density of pearl millet (Alka *et al*., 2012:67). These observations may also be a result of starch and peptide linkage hydrolysis suggested by Hallén *et al.* (2004:181) and Ikujenlola (2008:473). These qualities were also corroborated by Desikachar (1980), who prescribed fermentation as a traditional method for low-bulk weaning food manufacture.

2.1.3.4 Wet Cooking and Drying

Wet cooking is traditionally used to produce readily edible thick or thin porridges, which involves mixing grain flour with aliquots such as water and or milk, with salt and sugar added for taste (Legesse, 2013:33). The process normally involves boiling the slurry

with constant stirring until the desired consistency is reached. Cooking is highly dependent on the type of protein and kinetics of temperature and time. In general cooking or heat treatment results in proteins losing their complex configuration, exposing the peptide chain to hydrolytic enzymes and improving *in vitro* protein digestibility. However cooking at a very high temperature and or for extended periods leads to extensive denaturation of proteins, reducing the digestibility of gruel due to protein cross-linking through the disulphide bonds, and deterioration and degradation of amino acid residues (Joye, 2019:6; Pushparaj & Urooj, 2011:897). For instance, Abdelrahman *et al.* (2005:397), reported a 6.5% reduction of phytic acid and polyphenol content in the wet cooking of pearl millet thus reducing antinutritional factors.

Drying of porridges or gruels can be achieved through extrusion, cabinet drying, roller drying, foam mat drying and freeze-drying. Although not commercially viable, freeze-drying cereal gruels or porridges is very ideal for experimental processes as it is the least detrimental to nutrient content and quality. Freeze-drying can also accommodate cooked small experimental batches (van der Merwe, 2017:63). The process is normally followed by milling to attain desired particle size and improve dispersibility and reconstitutability.

2.2 Moringa oleifera

Moringa leaf powder (MLP) is made from the *Moringa oleifera* tree, a drought-tolerant plant that is natively from India although it is also cultivated in southern Africa (Mabapa *et al*., 2017:161). With a protein content of just over 30%, MLP is superior to most legumes (24–26%) except for soybean at 36% (El-Fatah *et al*., 2013:1065; Olalekan & Bosede, 2010:92).

2.2.1 Taxonomy, Climatic Requirements and Morphology

The Moringa tree grows mainly on sandy or gravelly riverbanks, where the soils are usually well-drained and low in organic matter with the water table in a range within its root depth. However, the moringa tree can thrive in loamy to heavy clays with pH ranges of between 5.5 and 8 (Parrotta, 1993:1; Gadzirayi & Mupangwa, 2014:18). The tree grows fairly in tropical and subtropical climates under very warm, humid and wet environments, and can thrive in extreme temperature conditions of between -1 and 48°C (Parrotta, 1993:1; Gadzirayi & Mupangwa, 2014:18). It can grow in low to high (300 to 3000 mm per annum) rainfall conditions with leaf yields equalling 30% of the total biomass harvested (Newton, 2006:2; Gadzirayi & Mupangwa, 2014:18).

2.2.2 Nutrient profile of moringa leaf powder

Table 2.3 shows a comparison of macronutrient and essential amino acid content of *Moringa oleifera* leaf powder (MLP) as reported by various researchers and the WHO standard requirements.

Table 2.3: Macronutrient and amino acid composition of MLP

Component	Quantity in MLP	WHO Standard Requirements[3]
Macronutrients (%)[1]		
Moisture	9.533 ± 0.194	-
Ash	7.64 ± 0.433	-
Crude fat	6.50 ± 1.042	-
Crude Protein	30.29 ± 1.480	-
Crude fibre	19.89	-
Essential Amino Acids (g/100 g protein)[2]		
Leucine	8.49	5.9
Isoleucine	4.15	3.0
Lysine	5.71	4.5
Methionine	1.88	2.2[a]
Phenylalanine	5.65	3.8[b]
Threonine	4.71	2.3
Valine	5.50	3.9
Histidine	2.92	1.5

1. Adapted from Moyo *et al*. (2011:12927)
2. Adapted from El-Fatah *et al*. (2013:1069)
3. Requirements pattern for adults WHO *et al*. (2007:135)
a. Methionine+cysteine
b. Phenylalanine + Tyrosine

The protein efficiency ratio and biological value of MLP are equally high and comparable to legumes (El-Fatah *et al*., 2013:1073). The amino acid profile of MLP meets and exceeds WHO standard requirements with lysine, the limiting one in cereals, at 5.87 g/100 g protein. Moreover, it is high in the sulphur-containing amino acids methionine and cysteine, which are deficient in pearl millet and other cereals. MLP also has a high fibre content at 16.2%, a substantial amount of fat (with a high proportion of omega-3 essential fatty acids) and low carbohydrate content at 36% (El-Fatah *et al*., 2013:1069; Moyo *et al*., 2011:12927).

Table 2.4 illustrates the micronutrient profile of MLP against WHO standards. The vitamin A, thiamin, and riboflavin content of MLP exceed daily requirements with niacin and vitamin C just above 50% and below 40% of daily requirements, respectively.

Table 2.4 Micronutrient composition of MLP

Components	**MLP**	***WHO RI/Day[2]**
Vitamins (unit/100 g)[1]		
Vitamin A (mg)	18.9	500 – 600 (mcg)
Thiamin (mg)	2.6	1.1 – 1.2
Riboflavin (mg)	20.5	1.1 – 1.3
Niacin (mg)	8.2	14 – 16
Vitamin C (mg)	17.3	45
Major Elements (units/100 g)[3]		
Calcium (mg)	2600	1000–1300
Phosphorus mg)	425	-
Magnesium (mg)	555	220–260
Potassium (mg)	1900	-
Sodium (Na)	28	-
Trace Elements (units/100 g)	4.07	
Iron (mg)	57.5	9.1–19.6[#]
Manganese (mg)	4.54	-
Zinc (mg)	2.49	4.7–7
Copper (mg)	0.951	-

*WHO Recommended intake for adults per day

#WHO Recommended intake at 15% bioavailability

1. Adapted from Fuglie (2001:172)

2. Adapted from FAO & WHO (2004:206)

3. Adapted from Melesse *et al*. (2011:3)

The calcium, magnesium and iron content of MLP covers just above 200% of daily requirements with substantial quantities of phosphorus, potassium and traces of zinc, manganese, sodium and copper. MLP, therefore, presents a good attribute of complementing the micronutrient profile of pearl millet and could alleviate 'hidden hunger' and account for its success in alleviation of malnutrition (Madukwe *et al*., 2013:226).

2.2.3 Nutraceutical benefits of MLP

Generally, Moringa leaves contain various antioxidants other than those of vitamin nature (ascorbic acid, flavonoids, carotenes and phenolics). The leaves are effective for treating or reducing the chances of occurrence of a wide spectrum of medical

conditions on both traditional and modern pharmaceutical platforms. MLP has also been identified as effective in the treatment of anaemia because of its protein quality, and substantial iron and vitamin C content (Madukwe *et al*., 2013:228). Toma & Deyno (2014:225), and Verma *et al.* (2012:47) found Moringa leaf extract to possess anti-ulcerogenic and hepatoprotective properties. Toma & Deyno (2014:226) also found Moringa leaves to possess notable serum cholesterol-lowering action attributed to β-sitosterol, while glycosides were found to lower blood pressure. A combination of these two and other diuretic properties render Moringa leaves crucial in preventing cardiovascular disorders. Other vital properties of Moringa leaves include anti-cancer, anti-tumour, anti-inflammatory and anti-hyperglycemic activities (Razis *et al*., 2014:8572; Toma & Deyno, 2014:226).

2.3 Food compositing and enrichment of cereals with legumes

Food compositing involves combining two different nutrient sources to produce a more nutrient-balanced product utilising the nutrient-complementary benefits of the individual foodstuffs as noted in protein enrichment of cereals with legumes (Nnam, 2001:251; Pelembe *et al*., 2002b:120; Anyango, 2009:29). Numerous composite foods have been developed, most notably in cereals utilising legumes (cowpea, chickpea, and pulses) to produce a significant nutrient improvement with minimal reduction in acceptability of the final food product (Pelembe *et al*., 2002b:125; Anyango, 2009:29; El-Fatah *et al*., 2013:1078).

Table 2.5 shows the nutritional impact of legumes on pearl millet and other common cereals. In separate studies, Modu *et al.* (2010:165) and Dlamini (2016:46) reported a 50% and 24.8% protein increase through compositing pearl millet and sorghum with cowpea flour, respectively. Dlamini (2016:48) reported a 98.4% increase in the lysine content of sorghum with a resultant 3.6 g/100 g covering 82% of diet requirements. The addition of cowpea to pearl millet resulted in slight increases in mineral content, particularly iron, zinc and calcium, whilst the necessity for improving the calcium and zinc content in sorghum was recommended (Dlamini, 2016:52). Chickpea seed powder yielded a 21.8% protein content increase when composited at 15% with wheat (El-Fatah *et al*., 2013:1075). This was accompanied by slight increases in mineral content although still far below the recommended intake. Soybean flour delivered a very significant increase (154%) in protein content accompanied by an equally high increase in fat when composited at 20% with maize (Edema *et al*., 2005:914). However, there were very slight increases in the mineral and vitamin content of maize.

Table 2.5 Enrichment of various cereals through compositing with legumes

Components	**Cereal Composites**			
	PM+CP[1]	W+CSP[2]	M+SB[3]	M+SB[4]
Macronutrients (g)				
Protein	13.26	12.35	22.76	12.17
Fat	2.60	12.73	8.66	2.15
Ash	1.37	1.48	2.88	1.00
Crude Fibre	2.90	1.69	2.88	2.17
Carbohydrates	75.50	71.75	57.58	72.80
Micronutrients (mg)				
Lysine*	4.99	-	-	3.69
Calcium	130.17	75.79	13.10	29.86
Iron	7.04	2.39	2.93	3.93
Zinc	85.03	13.44	-	1.66

*Unit per 100 g protein

PM+CP = 70% pearl millet flour + 30% cowpea flour; W+CSP = 85% wheat flour + 15% chickpea seed powder; M+SB = 70% Maize flour + 30% soybean flour; S+CP = 75% sorghum + 25% cowpea flour.

1. Adapted from Modu *et al*. (2010:165)
2. Adapted from El-Fatah *et al*. (2013:075)
3. Adapted from Edema *et al*. (2005:914)
4. Adapted from Dlamini (2016:46)

Table 2.6 shows the impact of Moringa leaf powder when composited at low proportions to wheat noodles in a study to produce instant noodles augmented with natural sources of high lysine content. The supplement levels of MLP at 5% and 7.5% improved the protein content by 10.5% and 15.4%, respectively. This was accompanied by an up to 92% ash content increase with calcium and iron increasing by 496% and 290% respectively. Zinc increased by 22.5% whilst magnesium increased from 0.34 mg to 42.69 mg. The colour, taste and texture, as well as overall acceptability of MLP-supplemented products, were better than the control (100% wheat) and quite comparable to chickpea seed powder-supplemented variants (El-Fatah *et al*., 2013:1078).

Table 2.6 Macronutrient composition and mineral content of noodles supplemented with moringa leaf powder (MLP) at different levels (on a dry weight basis).

Components	Formulations		
	Wheat (100%)	Wheat + MLP (5%)	Wheat + MLP (7.5%)
Micronutrients (g)			
Protein	10.14	11.2	11.7
Fat	12.00	12.21	12.32
Ash	1.00	1.61	1.92
Crude Fibre	1.15	2.01	2.40
Carbohydrates	75.21	72.97	71.66
Minerals (mg)			
Calcium	53.0	228.08	315.66
Iron	1.35	3.95	5.26
Zinc	0.80	0.92	0.98
Magnesium	0.34	28.58	42.69

Adapted from El-Fatah *et al*. (2013:1075)

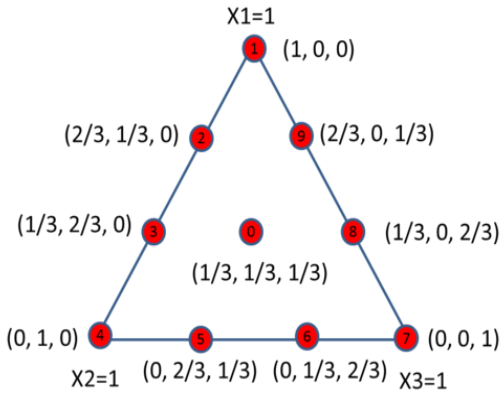
2.4 Mixture experiments

Mixture experiments are response surface experiments used in product design whereby the product under investigation is made up of several components (Wangkananon *et al*., 2018:179). In dual or multi-component mixtures experiments, it is key to understand the contributions of each of the components to the final mixture in terms of composition, functionality and or cost. The utmost goal of mixture design experiments is to find optimal proportions for each of the components that yield a highly desirable response for a selected variable. Statistical analysis and mixture design are employed synergistically to assess the effect of combining two or more components and to forecast the response(s) for all probable formulations of the mixture (Giese *et al*., 2011:239; Wangkananon *et al*., 2018:179; Goos *et al*., 2016:899). These two techniques are efficient for systematic projection and performing research by providing data or permutations that can be scrutinised to construct legitimate and factual inferences (Damiri *et al*., 2016:157).

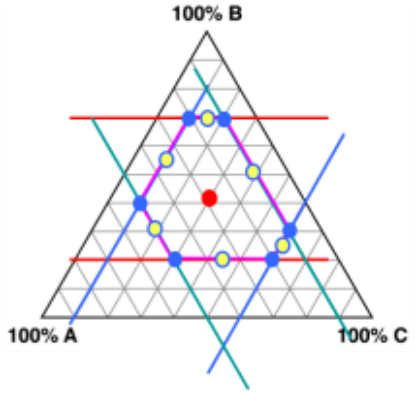
The standard mixture designs for fitting standard models comprise simplex-lattice, simplex-centroid, and extreme vertices designs and, in most instances, both upper and lower bound limits are set thus reducing the experimental space (Damiri *et al*., 2016:157). An extreme vertices mixture design is an experimental design utilised for the optimisation of an experimental mixture component proportions within a set of

detailed constraints or limits (Cleland & McCluskey, 2013:4673). It has simultaneously been used to explain the correlation between investigated variables and results and to facilitate the optimisation of several variables to obtain the best response, especially in the examination of food, beverage, and chemical manufacturing (Zhang *et al.*, 2015:5831).

Figure 2.1 shows two structures of the simplex and extreme vertex design plots with the simplex design indicating a mixture design in which the design points are set in a uniform way (or lattice) on an L-simplex ranging from 0 to 1 (100%). The extreme vertex design plot however shows experimental space constrained within lower and upper bounds thus covering only a sub-portion within the simplex to adequately cover the design space (Minitab, 2019).



Simplex Plot
(non-constrained mixture)



Extreme Vertex Design
(constrained mixture)

Figure 2.1 Mixture Design Plots.

2.5 Conclusion

Pearl millet is a vital grain and source of nutrients for large populations in the underdeveloped areas of Sub-Saharan Africa, India and China as it provides substantial quantities of essential amino acids, fatty acids, minerals and vitamins in the form of porridge or beverages. Malting and fermentation have been employed in improving the nutritional, rheological and organoleptic properties of pearl millet. Other efforts to improve its protein content to curb protein-energy malnutrition and boost its overall nutritional profile by compositing with legumes have been fairly successful although coupled with the introduction of antinutritional factors, toxic components and

flatulence causing raffinose family oligosaccharides. There is also a further need to address other micronutrient deficiencies widespread in Africa. Moringa leaf powder presents a great opportunity for improving the nutritional quality of pearl millet-based foods as it is high in protein and the essential amino acid lysine (deficient in pearl millet) accompanied by an exceptional overall nutrient balance including micronutrients. Moringa leaf powder also offers substantial phytochemicals to impart nutraceutical functionalities in pearl millet foods. For optimum results in compositing, response surface methodology such as mixture design may aid in finding optimal proportions for compositing to yield highly desirable outcomes.

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CHAPTER THREE

EFFECT OF FERMENTATION AND MALTING TIME ON PASTING PROPERTIES, BULK DENSITY, PROTEIN CONTENT AND ANTIOXIDANT PROPERTIES OF PEARL MILLET FLOUR

Abstract

Malting and fermentation have been long employed as traditional and industrial means of improving the nutritional, biochemical and physicochemical properties of various cereal grain flours and gruels. Some of the most notable benefits of the two processes comprise increased protein content, lowered viscosity pastes, reduced antinutritional factors and improved antioxidant properties of food products. This study examined the effect of fermentation and malting time on the protein content, pasting properties, bulk density and antioxidant properties of pearl millet (Babala variety). Fermentation was conducted by employing back-slopping, whereby a smaller batch of raw pearl millet flour slurry is prepared for spontaneous fermentation over a 24 h period at 37°C. Bulk fermentation was conducted by adding a 5% portion of the fermented slurry as a starter to a bigger batch of raw pearl millet slurry and incubating at 37°C for 36 h with subsequent freeze drying and milling. Malting was carried out by a three-stage process comprising a 3 h soaking step NaOH solution followed by germination in a proofing oven at 30°C and ~98% relative humidity for 36 h. The final step, kilning, involved drying the germinated seeds in a cabinet drier at 50°C for 48 h and subsequent milling. Samples were collected at 6 h intervals during the respective processes and results from the analyses were as follows. Final viscosity ranged from 597.67 to 1435.33 Cp and 55.00 to 866.67 cP during fermentation and malting, respectively. Bulk density ranged from 294.33 to 558.77 kg/m^3 and 511.00 to 587.15 kg/m^3 during fermentation and malting, respectively. The protein content ranged from 11.01 to 12.69 g/100 g and 9.21 to 11.12 g/100 g during fermentation and malting, respectively. Total phenolic content ranged from 284.67 to 460.51 mg GAE/100 g and 186.67 to 342.56 mg GAE/100 g during fermentation and malting, respectively. Oxygen radical absorbance capacity (ORAC), ranged from 8127.67 to 13225.00 µmol TE/100 g and 6530.00 to 8127.67 µmol TE/100 g during fermentation and malting, respectively. Ferric-reducing antioxidant power (FRAP) ranged from 1161.87 to 1391.48 µmol AAE/100 g and 989.67 to 1498.63 µmol AAE/100 g during fermentation and malting, respectively. Overall, fermentation and malting for 36 h respective periods increased the protein content and improved the pasting properties of pearl millet flour, respectively.

3.1 Introduction

Pearl millet flour is mostly used to make porridge or beverages for large populations, mainly in the underdeveloped areas of Sub-Saharan Africa, India and China. Staple to some regions, pearl millet gruels and beverages are sources of protein, energy and minerals, playing a vital part in fighting global food insecurity and alleviating malnutrition. Considering that pearl millet flour porridge is used as a weaning meal and generally has to compete with mainstream cereals (wheat, rice and maize) for taste, texture (viscosity) and acceptability, there is an opportunity to improve on these quality indicators (Yadav *et al*., 2014:2169). Bulk density is also another key parameter to be considered for pearl millet flour as it has implications on the determination of packaging options, materials handling, and application of wet processing (Murugkar *et al*., 2013:11). Pearl mill*et al*so contains substantial quantities of phenolic compounds with some possessing antioxidant capabilities beneficial to keeping quality and human health. However, some phenolic compounds present antinutritional functionalities by forming complexes or binding to nutrients (Bryden *et al*., 2013:13; Issoufou *et al*., 2013:503).

Malting, fermentation, or enzymatic treatment have been suggested to improve the nutritional, biochemical, physicochemical and functional properties (due to protein and carbohydrate structural modifications) of pearl millet (Issoufou *et al*., 2013:505). These processing techniques assist in the production of lower-viscosity pearl millet food products and the reduction of antinutritional factors thus improving nutrient bioavailability. The given benefits are carried over to the consumers such as weaning-stage infants, patients or even anyone (for example athletes) who require semi-liquid or low-viscosity meals (Pelembe *et al*., 2002a:7; Hallén *et al*., 2004:178; Coulibaly *et al*., 2011:6; Issoufou *et al*., 2013:503; Obilana *et al*., 2014:255).

This study examined the effects of fermentation and malting time on pasting properties, bulk density, protein content and antioxidant properties of pearl millet flour to identify optimal periods for the two respective processing techniques and their overall impact thereof.

3.2 Materials and Methods

3.2.1 Source of materials

Pearl millet grains were acquired from AGT Foods, Cape Town, South Africa. All chemicals were purchased from Merck (Pty) Ltd apart from sodium carbonate and gallic acid standard that were bought from Sigma-Aldrich (Pty) Ltd.

3.2.2 Fermentation of pearl millet

Fermentation was carried out as prescribed by Osman (2004:130) with slight modifications. Spontaneous fermentation was initiated by mixing a small batch of raw pearl millet flour with distilled water (1:2 w/v) in a 5 L plastic bucket and stored in an incubator (Memmert, Germany) at 37°C for 24 h. Samples were collected at 6 h intervals from the preparation of the starter slurry until 24 h and analysed for pH and total titratable acidity (TTA). A bulk fermentation slurry was then prepared using the same ratios of pearl millet flour to water, to which a portion of the fermented slurry, equivalent to 5% of the bulk unfermented mix, was added as a starter culture. This process is referred to as back-slopping and it provides active LABs to initiate bulk fermentation while shortening the time required to reach the desired pH or acidity (Sahlin, 1999:32; Osman, 2004:130).

The bulk fermentation was then carried out by incubating the inoculated slurry at 37°C for 36 h while samples were drawn every 6 h and analysed for pH and TTA. All samples were then freeze-dried and milled using a universal cutting mill with a 0.75 mm sieve (Pulverisette 19, Fritsch, Germany). The milled fermentation samples were then kept refrigerated at <5°C in sealed ziplock plastic containers until the point of use and or analysis for pasting properties, bulk density, antioxidant properties and protein content.

3.2.3 Malting of pearl millet

Pearl millet seeds were visually sorted for any foreign matter before winnowing to remove any excess lightweight particles. The clean grains were soaked in a 25 L plastic bucket at a 1:1.5 mass ratio of grain to the solution of 0.03% sodium hydroxide, which reportedly enhances enzyme activity at 25 $\pm$ 3°C for 3 h with periodic agitation. The caustic solution was drained before the washing of grains with clean running water and the subsequent draining of excess water.

The soaked pearl millet grains were spread on perforated plastic trays lined with a muslin cloth in layers of up to 2 cm. The trays were then packed onto a trolley with perforated pans and loaded into a proofing oven (Prover, Macadams, Cape Town) at 30°C and approximately 98% relative humidity. The germination process occurred for 36 h, with 12 hourly rinses in clean tap water to prevent mould growth. Samples were collected at 6 h intervals of germination from 18 h to 36 h and dried in a cabinet drier (Geiger & Klotzbucher, South Africa) at 50°C for 48 h with occasional turning over of grain, before milling. Milling of the dry grains was performed with a hammer mill (TRF 400, Metalurgica Trapp, Brazil) and sieved through a 2 mm mesh. The malted pearl millet flour samples were then sealed in ziplocked polypropylene bags and kept

refrigerated at <5°C until the point of use and or analysis for pasting properties, bulk density, antioxidant properties and protein content.

3.2.4 pH and total titratable acidity of fermenting pearl millet slurries

pH determination was done using a calibrated pH meter. The pH meter was standardised with buffers 4 and 7. An electrode was immersed into 20 ml of sample pre-weighed into a beaker and a reading was taken. Total titratable acidity (TTA) was measured by titrating 10 ml of sample with 0.1 M NaOH until pH 8.5 was reached, where pH was measured using a calibrated pH meter. TTA was expressed as a percentage of lactic acid (AOAC, 1980). The TTA was calculated as per Equation (3.1).

$$TTA\ (\%) = \frac{ml\ NaOH \text{ x } M\ NaOH \text{ x } M.E}{Volume\ of\ sample \text{ x } 1000} \text{ x } 100 \qquad \text{Equation (3.1)}$$

Where:

ml = Volume of NaOH (ml) used to reach pH 8.5

M = Molarity of NaOH

ME = Lactic acid equivalent factor being 90.08 mg

1000 = factor used to convert the M.E which is normally in mg to grams, and

100 = Conversion factor to %.

3.2.5 Pasting properties of fermented and malted pearl millet flours

Pasting properties were characterised using the Rapid Visco Analyzer (RVA 4500, Perten Instruments, Australia). Approximately 3 g of sample were mixed with 25 ml of distilled water and heating cycles were set as follows: the sample was heated to 50°C for 1 minute, with subsequent heating to 95°C over 3.42 mins and held for 2.5 mins at 95°C before cooling to 50°C over 3.80 mins and holding at this temperature for the final 2.5 mins with a total analysis time adding up to approximately 13 mins. The following parameters were analysed, peak viscosity (PV), holding strength or trough viscosity (TV), breakdown viscosity (BV), final viscosity (FV), setback viscosity (SV) and peak time (PT) and pasting temperature. All samples were analysed in triplicate.

3.2.6 Bulk density of fermented and malted pearl millet flours

Bulk density (BD) was measured according to the ASTM D7481-09 Method by weighing the mass of a powdered sample freely poured into a 100 ml graduated cylinder and expressed as weight per unit volume (kg/m^3) (ASTM, 2009:1). All measurements were done in triplicate and bulk density was calculated as per Equation (3.2).

$$BD\ (kg/m^3)\ = \frac{sample\ weight\ (g)\ \text{x}\ 0.001}{100\ ml\ \text{x}\ 0.000001}$$ Equation (3.2)

Where:

0.001 = Conversion factor from g to kg and 0.000001 = Conversion factor from ml to m^3.

3.2.7 Protein Content of fermented and malted pearl millet flours

Protein content was determined using the nitrogen analyser (TruSpec® N, Leco Corporation, MI, USA) using 6.25 as the nitrogen conversion factor.

3.2.8 Total phenolic content of fermented and malted pearl millet flours

Total phenolic content was measured using the Sadasivam & Manickam (1996:193) method, with minor modifications as per Obilana (2013:122) using a 96-well plate reader. A 1% HCl–methanol solution was prepared and used as an extraction solvent using a 1:10 (v/v) sample to the solvent ratio in 50 ml screw-cap tubes. Tubes were shaken gently to adequately mix components and left to stand overnight in a dark place before preparation for analysis.

Test reagents were prepared as follows: Ethanol (EtOH) 10% was prepared in a 1000 ml media bottle, by adding 100 ml of ethanol to 900 ml H_2O. Folin reagent was prepared in a 15 mL screw-cap tube, by adding 1 ml Folin-Ciocalteaus phenol reagent to 9 ml H_2O and mixing well. Sodium Carbonate (7.5%) was prepared in a 100 ml media bottle, by weighing 7.50 g Na_2CO_3 and adding 100 ml H_2O before being mixed until well dissolved. The gallic acid standard was prepared in a 50 ml screw-cap tube by dissolving 40 mg (0.040 g) of gallic acid in 50 ml 10% EtOH to give a stock standard concentration of 800 mg/l.

In 6 Eppendorf tubes, standard stock solutions of 0, 20, 50, 100, 250 and 500 mg/l were prepared by diluting with 10% EtOH. A 25 µl portion of each different concentration of the standard and extracts were pipetted into the 96 well plates in triplicate before adding 125 µl of the Folin-Coicalteau phenol reagent and the mixture was left to stand for 5 minutes. Afterwards, 100 µl of the 7.5% Na_2CO_3 solution was added to each well and the plate was left to stand for 2 h at room temperature. Absorbance in the wells was read at 750 nm using a spectrophotometer (Multiskan Spectrum, Thermo Electron Corp., Waltham, MA, USA)**.**

3.2.9 Oxygen radical absorbance capacity assay

Samples for the ORAC assay were prepared as per TPC extraction (3.2.7). Reagents were prepared as per the following. The preparation and analysis procedures were carried out as per the (Antioxidant Research Laboratory, 2006). Phosphate buffer 75 mM, pH 7.4 was prepared by weighing 1.035 g sodium di-hydrogen orthophosphate-1-hydrate ($NaH_2PO_4.H_2O$) in a 100 ml media bottle, with the subsequent addition of 100 ml ddH_2O and mixing until dissolved. A 1.335 g portion of disodium hydrogen orthophosphate dehydrate ($Na_2HPO4.2H_2O$) was weighed in a 100 ml media bottle and 100 ml ddH_2O was then added before mixing components until dissolved. An 18 ml of 1st solution was mixed with 82 ml of 2nd solution. Fluorescein sodium salt was prepared by dissolving 0.0225 g $C_{20}H_{10}Na_2O_5$ in a 50 ml phosphate buffer. The Trolox standard 500 µM stock solution was prepared by weighing 0.00625 g 6-Hydroxy-2,5,7,8-tetra-methylchroman-2-carboxylic acid in a 50 ml screw-cap tube and adding 50 ml phosphate buffer with Gilson pipetting aid with subsequent mixing until dissolution. A 150 mg portion of peroxyl radical, AAPH (2,2'-Azobis (2-methylpropionamidine) dihydrochloride was pre-weighed into a 15 ml screw cap tube.

The fluoroskan was switched on before starting the assay to allow the machine to reach a temperature of 37°C. The excitation wavelength and the emission wavelength were set at 485 nm and 530 nm, respectively. Six Eppendorf tubes were marked A–F and standard stock solution and diluents were added to each tube as described in Table 3.1.

Table 3.1 Trolox standard series

Tube	Standard concentration (µM)	Trolox stock solution (µl)	Phosphate Buffer (µl)	Well number
A	0	0	750	A1-A3
B	83	125	625	A4-A6
C	167	250	500	A7-A9
D	250	375	375	A10-A12
E	333	500	250	B1-3
F	417	625	125	B4-6

The 96-well plate reader was loaded with Trolox standards, Control and Sample wells. A 10 µl portion of the fluorescein stock solution was added into 2 ml phosphate buffer in an Eppendorf tube and 240 µl of this solution was diluted in 15 ml phosphate buffer using a 15 ml screw cap tube. A 138 µl portion of this solution was pipetted with a multichannel pipette into each well of a black 96-microwell plate. A 6 ml

portion of the phosphate buffer was added to the pre-weighed AAPH and mixed well until dissolved. Lastly, 50 µl of this solution was transferred using a multichannel pipette into each well. The multiwell plate was inserted into the fluorometer and analysis was initiated.

The results were based on the principle that one ORAC unit is assigned as being the net protection area provided by 1 µM Trolox in the final concentration. The area under the curve for the sample was compared to the area under the curve for Trolox, and the result given was reported in Trolox equivalents per weight of the sample (µmol TE/100 g). Equation (3.3) shows a summary of the calculation methodology.

$$ORAC\ Value\ = \frac{Area\ under\ sample\ curve - area\ under\ blank\ curve}{Area\ under\ Trolox\ curve - area\ under\ blank\ curve} \quad \text{Equation (3.3)}$$

3.2.10 Ferric reducing antioxidant power (FRAP)

The FRAP assay is fundamentally based on an oxidation/reduction reaction to measure the ability of a sample to reduce Fe^{3+} to Fe^{2+} whereby an antioxidant donates electrons in the same manner as a reductant in an oxidation/reduction. The ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex is often used in this reaction whereby its reduction, at low pH, produces the blue-coloured ferrous tripyridyltriazine (Fe^{2+}-TPTZ) form. The change in absorption at 593 nm is determined and then directly related to the reducing power of the electron-donating antioxidants in the food. FRAP, in this regard, is applied in food analysis to measure antioxidant capacity. Samples for the FRAP assay were extracted as per TPC extraction (3.2.7) and reagents were prepared as follows. Acetate buffer was prepared in a 1000 ml media bottle by adding 1.627 g sodium acetate and 16 ml glacial acetic acid before filling it up to the 1-litre mark with distilled water. Dilute HCL (40 mM) was prepared by adding 1.46 ml 32% HCl in a 1-litre media bottle before filling it up to the litre mark with distilled water. TPTZ ((2,4,6-tri[2-pyridyl]-s-triazine) was prepared by adding 0.0093g and 3 ml of 40 mM HCl to a 15 ml tube. Iron (III) was prepared in a 15 ml conical tube by adding 0.054 g $FeCl_3.6H_2O$ and distilled water. Prepared 1mM and 400µM L-Ascorbic acid were used as standard and control, respectively.

The multiskan spectrum plate reader was switched on 30 mins before taking readings. The FRAP reagent was prepared by adding 30 m acetate buffer, 3 ml TPTZ, 3 ml $FeCl_3$, and 6.6 ml distilled water in a 50 ml conical tube. The multiskan was set to 593 nm. In 6 Eppendorf tubes, standard stock solutions of, 0, 50, 100, 200, 500 and 1000 µM were prepared by diluting them with distilled water. A quantity of 300 µl FRAP

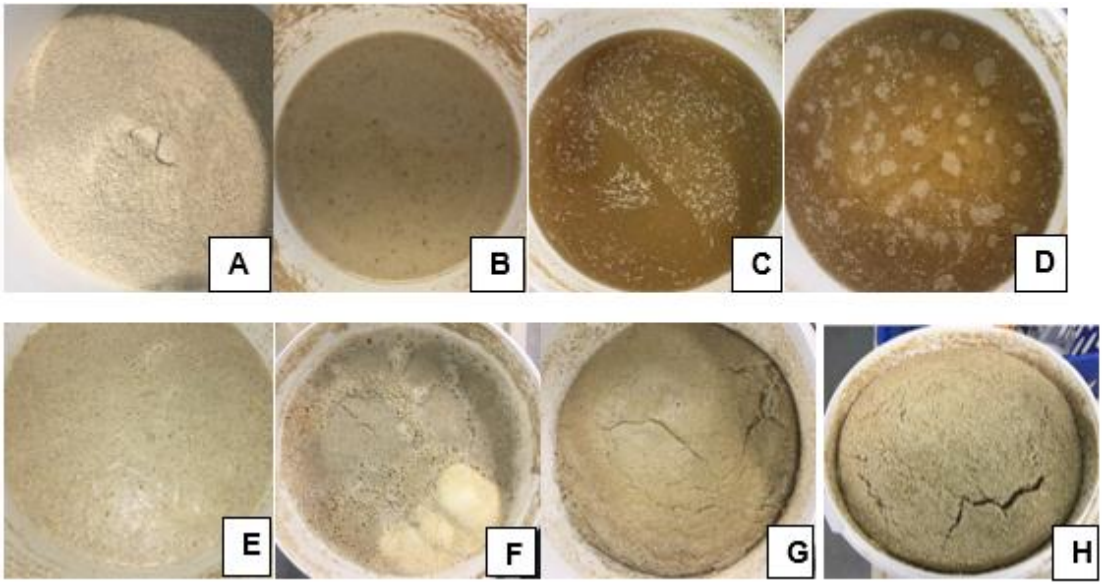
reagent was added to standards, controls and sample-containing wells using a multi-channel pipette incubation for 30 mins at 37°C before reading absorbance at 593 nm using a spectrophotometer (Multiskan Spectrum, Thermo Electron Corp., Waltham, MA, USA). Results were reported in Ascorbic acid equivalent per weight of the sample (AAE/100 g).

3.2.11 Data Analysis

Multivariate analysis of variance (MANOVA) was used to determine the mean difference between treatments and compositions (at p = 0.05) while Duncan's multiple ranges testes were employed to separate means where differences exist. To facilitate that, data or results were processed, trended, and analysed using the IBM Statistical Package for Social Science (IBM SPSS, version 26, 2019).

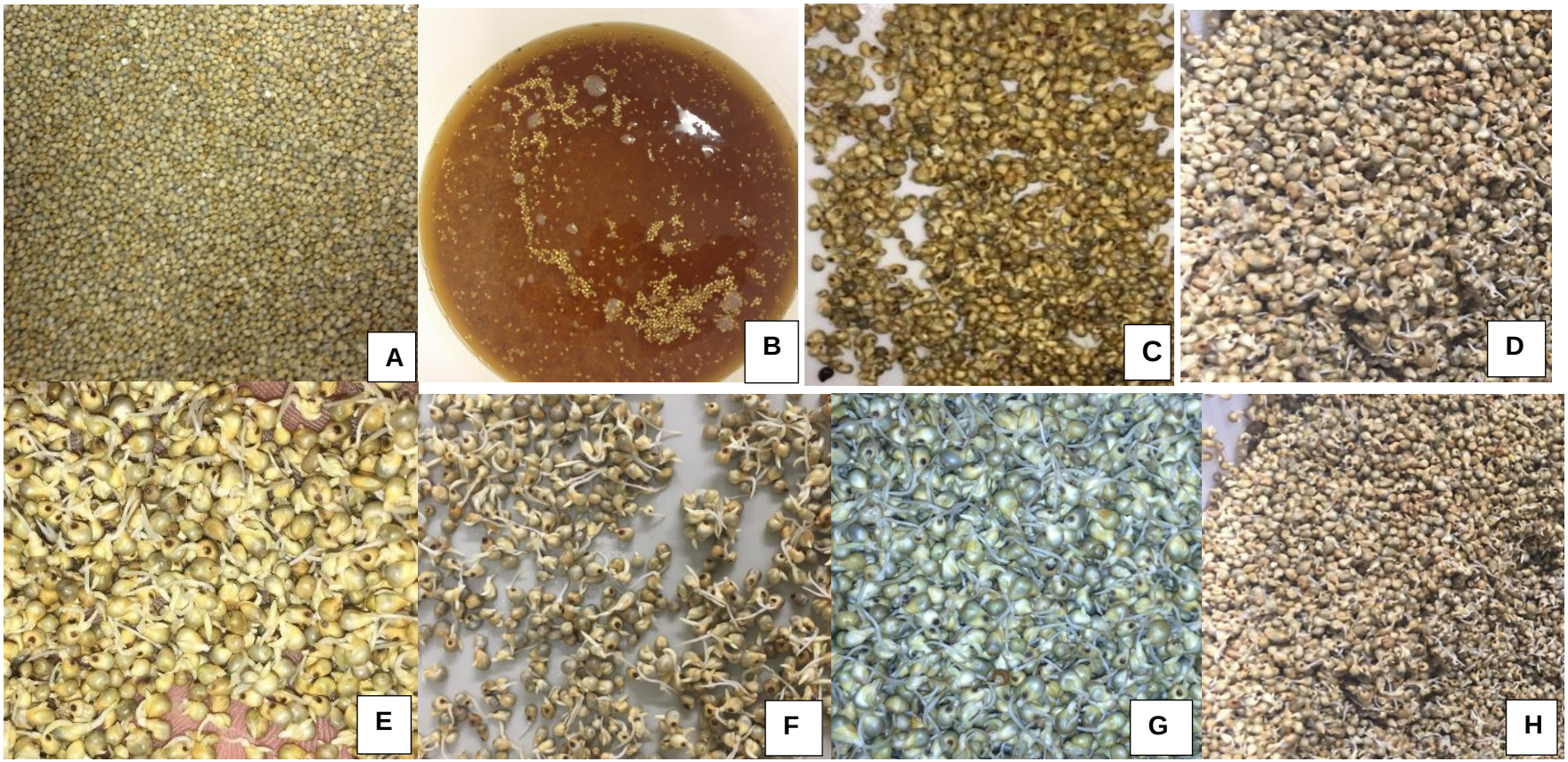
3.3 Results and Discussion

Figure 3.1 and Figure 3.2 show the visuals of pearl millet flour and pearl millet grains during different fermentation and malting intervals, respectively.



[A = Pearl millet flour, B = Pearl millet flour slurry, C = fermented pearl millet (6 h), D = fermented pearl millet (12 h), E = fermented pearl millet (18 h), F = Fermented pearl millet (24 h), G = Fermented pearl millet (30 h), H = fermented pearl millet (36 h).]

Figure 3.1 Stages throughout the fermentation process of pearl millet.



[A = Raw Pearl Millet grains, B = Steeped pearl millet, C = Germinated pearl millet (6 h), D = Germinated pearl millet (12 h), E = Germinated pearl millet (18 h), F = Germinated pearl millet (24 h), G = Germinated pearl millet (30 h), G = Germinated pearl millet (36 h).]

Figure 3.2 Pearl millet grain changes across malting stages (steeping and germination).

3.3.1 Effect of fermentation time on pH and titratable acidity (TTA) of pearl millet slurry

In the starter slurry preparation, the pH of raw pearl millet paste started at 6.35 and decreased to 4.79 over a 24 h period. The first 6 h during this phase only yielded a 0.10 drop in pH. This could be due to the lag phase of microbial growth as bacteria prepare for reproduction which is accompanied by the synthesis of DNA, inducible or adaptive enzymes and macromolecules that are required for cell division.

Table 3.2 shows the kinetics of pH and titratable acidity during the starter slurry fermentation and bulk fermentation slurry inoculated with 5% starter slurry. The pH of the unfermented starter slurry was 6.35 and comparable to the 6.37 reported by Jideani *et al.* (2021:6) for raw pearl millet slurry. pH dropped slightly within the first 6 h but started showing mild and steady dipping in the last 18 hours, with a final pH of 4.79 at 24 h. The bulk fermentation slurry inoculated with starter slurry had a starting pH of 6.01 and dropped to just under 4.79 within 12 h of fermentation, indicating the effectiveness of back-slopping in providing a relatively stable process and accelerating the fermentation process as recommended by Osman (2011b:2) and Franz *et al.* (2014:87).

Table 3.2 Effect of fermentation time on pH and TTA of pearl millet slurry[1]

	Starter Slurry (pH)	Bulk Fermentation (pH)	Bulk Fermentation (TTA %)
0	6.35 ± 0.01^{a}	6.01 ± 0.01^{a}	0.17 ± 0.01^{a}
6	6.25 ± 0.02^{b}	5.07 ± 0.02^{b}	0.49 ± 0.03^{b}
12	5.87 ± 0.02^{c}	4.71 ± 0.02^{c}	0.86 ± 0.03^{c}
18	5.38 ± 0.02^{d}	4.59 ± 0.01^{d}	1.17 ± 0.15^{d}
24	4.79 ± 0.01^{e}	4.58 ± 0.01^{d}	1.37 ± 0.08^{e}
30	ND	4.57 ± 0.03^{d}	1.61 ± 0.02^{f}
36	ND	4.57 ± 0.03^{d}	1.97 ± 0.05^{g}

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$). TTA – Titratable acidity, ND – Not done.

In bulk fermentation, the pH trend between 0 h and 12 h, characterised by a sharp drop in pH from 6.01 to 5.07 within 6 h, and to 4.71 after 12 h, aligns with the exponential growth phase of bacteria of the microbial growth curve where LAB cell division is normally rapid. The pH then dropped steadily to 4.59 at 18 h with no subsequent significant ($p \leq 0.05$) change observed for the remainder of the fermentation process as also observed by Jideani *et al.* (2021:6). The pH drop trend

was consistent with the results of Onuoha *et al.* (2017:14) who achieved comparable values of 4.80 and 4.55 after extended fermentation periods of 48 h and 96 h respectively. The steep pH drop during this period was due to the accumulation of lactic acid indicated by the simultaneous increase in TTA. Total titratable acidity increased consistently and significantly throughout the bulk fermentation process starting at 0.17% and peaking at 1.97% lactic acid equivalent over the 36 h period. The starting TTA value of 0.17% was similar to the values obtained by Owheruo *et al.* (2019:478) and Jideani *et al.* (2021:6) who both started with a TTA of 0.14% but yielded lower values of 0.69% and 0.18%, respectively after an extended fermentation period of 72 h. Similarly, Jideani *et al.* (2021:6) started with a TTA of 0.12% but yielded a lower final value of 0.56% after 36 h of pearl millet slurry fermentation. These trends may be an indication of the impact of back-slopping in triggering an early exponential growth phase and consequently an increased rate of acid production.

Overall, the lag phase in bulk fermentation was seemingly drastically shortened as active LABs, inducible enzymes and macromolecules required for cell division were introduced by the initial fermented sample added as starter culture (back-slopping). After 36 h, the pH of the pearl millet slurry dropped to 4.57 and this result was not significantly lower than the 4.58 achieved after just 18 h of bulk fermentation. This trend indicates that, with back-slopping, the fermentation period could be shortened to 18 h to achieve optimum pH, a time also recommended by Jideani *et al.* (2021:18) On the other hand, TTA continued to increase consistently and significantly as fermentation progressed, reaching 1.97% at 36 h of fermentation. This can be attributed to microorganisms' continuous action of converting available sugars into organic acids primarily lactic, acetic, and pyruvic acids.

3.3.2 Effect of fermentation and malting time on pasting properties of pearl millet

Table 3.3 and Table 3.4 show the pasting properties of pearl millet flour during the fermentation and malting stages, respectively.

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Table 3.3 Effect of fermentation time on pasting properties of pearl millet flour[1]

Time (h)	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)	Pasting Temp. °C	Peak Time (min)
0	281.67 ± 0.57^{a}	260.00 ± 2.00^{a}	21.67 ± 2.08^{a,c}	866.67± 17.62^{a}	606.67 ± 15.63^{a}	89.93 ± 0.03^{a,d}	5.00 ± 0.00^{a}
6	375.67± 18.90^{b}	338.00 ± 15.13^{b}	37.67 ± 3.79^{b}	597.67 ± 30.07^{b}	259.67 ± 15.04^{b}	90.95 ± 0.43^{b}	5.38 ± 0.10^{b}
12	761.50 ± 21.50^{c}	729.00 ± 20.53^{c}	32.50 ± 5.13^{c}	1228.00 ± 39.11^{c}	499.00 ± 18.58^{c}	88.18 ± 0.03^{a,c}	5.56 ± 0.10^{c}
18	482.00 ± 9.85^{d}	254.75 ± 9.54^{d}	23.50 ± 1.73^{a,c}	846.00 ± 20.50^{a}	387.50 ± 11.06^{d}	89.28 ± 0.49^{d}	5.42 ± 0.04^{b}
24	604.00 ± 23.43^{e}	362.40 ± 23.46^{e}	19.67 ± 4.04^{a}	973.33 ± 35.23^{d}	389.00 ± 11.79^{d}	89.17 ± 0.46^{d}	5.56 ± 0.10^{c}
30	532.67± 14.57^{d}	314.40 ± 17.00^{d}	28.33 ± 3.21^{c}	832.00 ± 27.51^{a}	327.67 ±10.50^{a}	89.42 ± 0.58^{d}	5.38 ± 0.04^{b}
36	830.00 ± 83.26^{f}	415.17 ± 75.90^{f}	27.00 ± 7.81^{a,c}	1435.33 ± 136.08^{e}	632.33 ± 60.58^{a}	87.85 ± 0.57^{c}	6.95 ± 0.04^{d}

[1]Values are mean ± standard deviation. Means with a different superscript in each column differ significantly ($p \leq 0.05$)

Table 3.4 Effect of malting time on pasting properties of pearl millet flour[1]

Time (h)	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)	Pasting Temp. °C	Peak Time (min)
0	281.67 ± 0.58^{a}	260.00 ± 2.00^{a}	21.67 ± 2.08^{a}	866.67 ± 17.62^{a}	606.67 ± 15.63^{a}	89.93 ± 0.03	5.00 ± 0.00^{a}
18	57.33 ± 2.31^{b}	50.67 ± 1.15^{b}	6.67 ± 1.15^{b}	111.33 ± 3.21^{b}	60.67 ± 3.79^{b}	DNP	4.69 ± 0.03^{b}
24	37.00 ± 1.00^{c}	29.67 ± 0.58^{c}	7.33 ± 0.58^{b}	56.67 ± 2.52^{c}	27.00 ± 2.00^{c}	DNP	4.04 ± 0.25^{c}
30	44.67 ± 1.15^{d}	29.67 ± 0.58^{c}	15.00 ± 1.00^{c}	64.33 ± 2.08^{c}	34.67 ± 1.53^{c}	DNP	4.42 ± 0.04^{b}
36	41.33 ± 5.51^{c,d}	27.33 ± 3.21^{c}	14.00 ± 2.65^{c}	55.00 ± 5.20^{c}	27.67 ± 2.08^{c}	DNP	4.02 ± 0.28^{c}

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$). DNP – Did not paste

During fermentation, peak viscosity (PV), trough viscosity (TV) breakdown viscosity (BV), final viscosity (FV), setback viscosity (SV), pasting temperature, peak time (PT) of pearl millet flours ranged from 375.67 to 830.00 cP, 338.00 to 729.00 cP, 19.67 to 37.67 cP, 597.67 to 1435.33 cP, 259.67 to 632.33 cP, 87.85 to 90.95°C, and 5.38 to 6.95 minutes respectively. The PV increased as fermentation time increased, with a significant ($p \leq 0.05$) drop noted at the 18 h mark, followed by a steady increase to a final PV of 830 cP from a starting PV of 281.67 cP for raw pearl millet flour (RPMF). Akinola *et al.* (2017:4) also corroborated this trend however reporting a lower final increase of 56% after 72 h of pearl millet spontaneous fermentation.

The BV initially climbed to 37.67 cP from 21.67 cP (RPMF) before gradually dropping to 19.67 cP at 24 h. This was followed by a notable increase to a final value of 27.00 cP after 36 h of fermentation, a value that is higher than the 19.82 cP reported by Akinola *et al*. (2017:4). The ability of flour (starch) to form a viscous paste is referred to as a final viscosity. The FV, an indication of the ability of flour (starch) to form a viscous paste, had an erratic dropping and increasing trend throughout the entire fermentation period starting at 866.67 cP of RPMF and peaking at 1435.33 cP after 36 h. The overall increase in viscosity observed during fermentation is thus a concern considering that it could be an indication of the need for a higher liquid ratio to solids upon cooking the gruel thus consequently reducing nutrient density in the final product. Set back viscosity dropped significantly ($p \leq 0.05$) from 606.67 cP (RPMF) to 259.67 after 6 h, then almost doubled at the 12 h point before dropping steadily to 327.67 cP and eventually rising to 632.33 cP after 36 h of fermentation. The drop in SV could be attributed to the reduction of amylose content due to microbial enzyme hydrolysis into simple fermentable sugars. The final SV value after 36 h of fermentation did not however match the findings of Akinola *et al.* (2017:5) who reported a decrease of 19% after 72 h.

The pasting temperature hardly fluctuated throughout the entire fermentation period averaging 89.16°C, a result that is similar to 89.60°C from Gull *et al.* (2016:99) and far lower than the 96.50°C reported by Akinola *et al.* (2017:5) after a longer fermentation period of 72 h. Generally, a lower pasting value indicates a lower water-binding capacity, reduced gelatinisation tendency and higher swelling property of starch meaning that the pearl millet gruel will start paste formation at a lower cooking temperature and possibly reduce the cooking period (Akinola *et al*., 2017:5; James *et al*., 2018:788; Iskakova *et al*., 2019:3983). There was however a significant ($p \leq 0.05$) difference in PT between RPMF and fermented PM flour at 36 h. Overall, an 18 h fermentation period gave optimum results considering the low PV, TV, BV and FV values.

During malting, PV, TV, BV, FV, SV, and PT ranged from 37.00 to 57.33 cP, 27.33 to 50.67 cP, 6.67 to 15.00 cP, 55.00 to 111.33 cP, 27.67 to 60.67 cP and 4.02 to 4.69 minutes respectively. PV dropped as malting progressed starting at 281.67 cP for RPMF to 57.33 cP within the first 18 h of malting, reaching 41.33 cP after 36 h, a final result which is approximately comparable to 42.33 cP reported by Obilana (2013:105). The decrease in peak viscosity to starch degradation and the formation of non-polar structures during protein-starch interaction during germination. Breakdown viscosity dropped significantly ($p \leq 0.05$) from 21.67 cP to a low of 6.67 cP after 18 h, the subsequent trend showed a steady rise to a final 14.00 cP.

Furthermore, malting yielded a substantial drop in both FV and SV, from 866.67 cP to 55.00 cP and 606.67 cP to 27.67 cP, respectively. Overall, malted pearl millet had the lowest values for all parameters assessed compared to fermented and raw pearl millet. Similar findings were reported by Obilana (2013:105) and Akinola *et al.* (2017:5) who attributed the trend largely to starch degradation during germination by amylase. The degradation of starch leaves the granules vulnerable and prone to breaking down during heating and mechanical agitation or shear stress and thus rendering the flour easier to cook into a gruel. The reduction of viscosity during malting was favourable as it translates to increased solids concentration and consequently higher nutrient density, as corroborated by Almeida-Dominguez *et al.* (1993:17). It is therefore fitting to indicate a 24 h period of malting as optimum for achieving low viscosity as most parameters remained unchanged for the rest of the period except for BV, which was almost 50% lower than the final 36 h value of 14.00 cP. Overall, malting improved pearl millet flour's pasting properties significantly ($p \leq 0.05$) thus partially confirming the third hypothesis which proposed that both malting and fermentation would improve the pasting properties of pearl millet flour.

3.3.3 Effect of fermentation and malting time on the bulk density of pearl millet flour

Table 3.5 shows the effects of both fermentation and malting on the bulk density of pearl millet flours, respectively.

Table 3.5 Effect of fermentation and malting time on the bulk density of pearl millet flour on a dry basis.[1]

Time (h)	Bulk Density (kg/m^3)	
	Fermented pearl millet	Malted pearl millet
0	558.77 ± 4.05^a	558.77 ± 4.05^a
6	325.73 ± 1.64^b	ND
12	319.15 ± 0.49^c	ND
18	312.65 ± 3.32^d	587.15 ± 3.04^b
24	310.40 ± 2.42^d	511.00 ± 1.41^c
30	294.33 ± 6.47^e	548.90 ± 0.14^d
36	467.07 ± 3.89^f	559.27 ± 4.80^a

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$). ND – Not done.

The bulk density of pearl millet decreased significantly ($p \leq 0.05$) with an increase in fermentation time reaching a low 294.33 kg/m^3 from 558.77 kg/m^3 after 30 h. This was followed by a sharp increase to the final value of 467.07 kg/m^3 at 36 h, equating to about a 16% overall reduction. This trend was in line with and comparable to the trends in Alka *et al.* (2012:68) and Akinola *et al.* (2017:4) where 18% and 22% overall reduction in bulk density in the fermentation of pearl millet were respectively reported. James *et al.* (2018:786) achieved a lower value of 430.00 kg/m^3 after 48 h of pearl millet fermentation citing that a lower bulk density promotes the digestibility of the product (infant formula) that is preferred for immature digestive systems. The ideal fermentation period according to the trend observed is therefore 30 h, where the lowest value of 294.33 kg/m^3 was achieved, equating to a 47% reduction in bulk density. Malting time had a fluctuating effect across the entire 36 h period reaching the lowest value of 511.00 kg/m^3 after 24 h, with a subsequent increase to 559.27 kg/m^3 after 36 h, a value that is not significant ($p \leq 0.05$) different to raw pearl millet flour's bulk density. Akinola *et al.* (2017:4) also reported no significant difference between the bulk density of raw and malted pearl millet flour.

The bulk density of flour is the overall function of particle density, interstitial and occluded air as well as the porosity of the flour. The high bulk density of flour may, as such, be attributed to the high particle density and low interstitial air and occluded air content observed in it (Rasane *et al*., 2015:3231). In industry, bulk density is an indication of the flour heaviness is also key in deciding the packaging requirements with other researchers citing that an increase in bulk density offers a

packaging advantage, as well as improved texture and mouthfeel (Akinola *et al*., 2017:4; Awolu, 2018:250).

3.3.4 Effect of fermentation and malting time on the protein content of pearl millet

Table 3.6 shows the effect of fermentation and malting time on the protein content of pearl millet, respectively.

Table 3.6 Effect of fermentation and malting time on the protein content of pearl millet flour[1]

Time (h)	Protein (g/100 g)	
	Fermented pearl millet	Malted pearl millet
0	11.12 ± 0.30^{a}	11.12 ± 0.30^{a}
6	11.42 ± 0.35^{a}	ND
12	11.01 ± 0.22^{a}	ND
18	12.41 ± 0.24^{b}	9.69 ± 0.60^{b}
24	12.66 ± 0.60^{b}	10.08 ± 1.01^{a,b}
30	12.69 ± 0.60^{b}	9.21 ± 0.45^{b}
36	12.40 ± 0.33^{b}	10.27 ± 0.46^{a,b}

[1]Results are reported as Mean ± Standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$). ND – Not done.

The first 12 h of fermentation resulted in no significant changes in protein content, a trend similar to the one of Osman (2011:3). At 18 h there was a significant ($p \leq 0.05$) increase in protein content with further fermentation resulting in no significant changes. Overall, there was an increase of 11.5% in protein content over 36 h of fermentation. This result is slightly lower than the ones provided by Kumari et al. (2022:6) and Ojokoh et al. (2015:4) who reported a minimum increase of 17% with 19%, respectively while Akinola et al. (2017:4) reported an elevated 24% increase. The increase in protein has been attributed to the loss of dry matter, primarily carbohydrates and an increase in amino acids due to the action of microorganisms (Nkhata et al., 2018:2450; Kumari et al., 2022:7). The optimum protein content was achieved at the 30 h interval, although there was seemingly no significant ($p \leq 0.05$) change after 18 h of fermentation.

During malting, the protein content of pearl millet significantly ($p \leq 0.05$) dropped after 18 h and was followed by slight up and down insignificant ($p \leq 0.05$) changes until 36 h. The decrease in protein during the early malting period has been

attributed to the leaching of low molecular weight nitrogen-containing compounds throughout the steeping process and rinsing of the grains (Pelembe *et al*., 2003). The overall effect of malting time on protein content was not significant ($p \leq 0.05$) considering the 11.12 g/100 g for raw pearl millet and the final figure of 10.27 g/100 g at the end of germination. This result is aligned with the findings by Obilana (2013:70) who also reported no significant changes in protein content after 36 h. On the contrary, Adebiyi *et al.* (2017:213) and Obadina *et al.* (2017:4445) reported, after 36 h of malting, a protein content increase of 22% and 16%, respectively. Furthermore, Obilana (2013:70), Iswarya & Narayanan (2016:393) and Owheruo *et al.* (2019:479) reported elevated protein content increases of between 25 to 56% after an extended malting period of 72 h. The effect of malting on protein has been reported to be conflicting with some scholars indicating increases and some decreases, based on the type of seeds. Increases in proteins are linked to the synthesis of some amino acids whilst decreases occur due to degradation by proteases hence the actual protein content being a result of the net effect of synthesis and breakdown (Nkhata *et al*., 2018:2453). It is therefore advisable to have an extended malting period of between 60 and 72 h to allow for optimal protein synthesis and substantial protein content increase.

3.3.5 Effect of fermentation and malting time on the total phenolic content (TPC) of pearl millet

Table 3.7 shows an overview of the effect that fermentation and malting had on the TPC of pearl millet flour.

Table 3.7 Effect of fermentation and malting time on the total phenolic content of pearl millet flour[1]

Time (h)	TPC[2] (mg GAE/100 g)	
	Fermented pearl millet	Malted pearl millet
0	284.67 ± 9.02[a]	284.67 ± 9.02[a]
6	425.13 ± 8.56[b]	ND
12	368.21 ± 10.88[c]	ND
18	412.83 ± 6.53[b]	231.28 ± 13.05[b]
24	417.44 ± 3.08[b]	186.67 ± 0.00[c]
30	460.51 ± 4.62[d]	251.29 ± 6.52[d]
36	342.57 ± 29.57[e]	342.56 ± 0.07[e]

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly. ($p \leq 0.05$). [2]TPC – Total phenolic content (GAE – Gallic acid equivalent). ND – Not done.

Fermentation ultimately yielded an increase in TPC, from a starting 284.62 mg/100 g comparable to 294 mg/100 g reported by Elyas *et al.* (2002:194) for RPMF, peaking at 460 mg/100 g at 30 h. There was however a significant ($p \leq 0.05$) consequent decrease in TPC to a final value of 342.56 mg/100 g after 36 h, although it was still significantly ($p \leq 0.05$) higher than that of RPMF. This trend was contrary to the findings of Elyas *et al.* (2002:194) who reported a substantial decrease in TPC as fermentation progressed, despite a similar irregular increase and decrease between the 20 and 36 h period. The increase in TPC due to fermentation was however corroborated by Kumari *et al.* (2022:8) who attributed the effect to higher enzyme activity. The increase in TPC occurs due to the release of bound phenolics into the matrix as endogenous hydrolytic enzymes introduced by LABs break down the cell wall and distort chemical bonds, hence liberating phenolic compounds. The subsequent reduction in TPC may be possibly attributed to polyphenol oxidase and other hydrolases degrading the liberated, bioactive phenolic compounds (Dhankher & Chauhan, 1987:828; Elyas *et al*., 2002:195; Adebo & Medina-Meza, 2020:5). The overall TPC range corresponded to the 304 to 444 mg/100 g for fermented and dehulled pearl millet variants that were reported by El Hag *et al.* (2002:194). In conclusion, a 36 h fermentation period was found ideal for optimum TPC yield.

The progression of malting was characterised by a significant ($p \leq 0.05$) initial drop in TPC starting at 284.62 mg/100 g and reaching a low 188.72 mg/100 g at the 24 h point. The speculation of steeping accompanied by rinse cycles in the initial stages of malting leading to the leaching of polyphenols could be correlated to this trend (Obilana, 2013:144). Adebiyi *et al.* (2017:214) also reported a decrease in total phenols during malting and attributed this effect to the activity of phenol oxidase catalysing the oxidation and conversion of phenols. There was however a significant ($p \leq 0.05$) overall increase to 342 mg/100 g in TPC after 36 h of malting, a result observed by Obilana (2013:148) in the malting of Babala pearl millet. Pradeep & Guha (2011:1644) and Owheruo *et al.* (2019:480) also reported higher TPC in germinated millet. Preedy (2014:20) and Carvalho *et al.* (2016:929) reported that malting resulted in the increase of free phenolics, which led to easier extractability, a phenomenon that could be correlated with the overall increase in TPC of malted pearl millet samples.

The increase in free phenolic compounds in millet seeds is reported to be due to cell wall-degrading enzymes, that are activated during germination thereby modifying the cell wall structure of the grain. The process results in the liberation of phenolic compounds such as ferulic and p-coumaric acids as the enzymes, most notably esterases, disrupt the ester and ether associations of these compounds with

the non-starch polysaccharides in the grain cell walls (Sharma *et al*., 2016:65). Overall, the ideal malting period for optimum TPC was found to be 24 h.

Overall, the optimal TPC phenomenon is a subjective matter. To elaborate, a proportion of the phenol compounds in millets may potentially be antinutrients, for example, phytates and phytic acid, polyphenols, tannins and goitrogens (Bryden *et al*., 2013:13). On the other hand, a substantial quantity of phenols in millets have been reported to present some potentially chemo-preventive functions and suggested to possibly prevent cardiovascular and neurodegenerative diseases associated with oxidative stress (Issoufou *et al*., 2013:504; Chandrasekara & Shahidi, 2011:435).

3.3.6 Effect of fermentation and malting time on the oxygen radical absorbance capacity (ORAC) of pearl millet flour

Table 3.8 shows the effects of fermentation and malting times on the ORAC (Oxygen radical absorbance capacity) of pearl millet.

Table 3.8 Effect of fermentation and malting time on oxygen radical absorbance capacity (ORAC) of pearl millet flour[1]

Time (h)	ORAC[2] (µmol TE/100 g)	
	Fermented pearl millet	Malted pearl millet
0	8127.67 ± 709.26[a]	8127.67 ± 709.26[a]
6	12621.00 ± 653.53[b,d]	ND
12	12340.00 ± 1561.29[c]	ND
18	13225.00 ± 968.74[b,d]	7524.00 ± 345.07[a]
24	11881.00 ± 1073.07[b,d]	6530.00 ± 405.88[a]
30	12917.33 ± 133.51[b,d]	7000.50 ± 303.35[a]
36	8959.33 ± 292.99[a]	7167.67 ± 1209.27[a]

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$)., [2]ORAC – Oxygen radical absorbance capacity (TE – Trolox equivalent). ND – Not done.

Fermentation resulted in no significant ($p \leq 0.05$) increase in the ORAC value of pearl mill*et al*though there was a drop and an increase noted throughout the fermentation period ranging between 8127.88 µmol TE/100 g for RPMF and 13282.56 µmol TE/100 g at 18 h of the fermentation period. The ORAC value dropped significantly ($p \leq 0.05$) between 30 to 36 h from 12917.25 to 8959.10 µmol TE/100 g respectively. The sporadic trend of ORAC values during fermentation positively correlated with the

TPC trend as per the findings of Chandrasekara & Shahidi (2012:6), Zhang *et al.* (2017:6) and Sindhu & Radhai (2018:138) with an optimal result achieved at 18 h.

Malting time had no significant ($p \leq 0.05$) impact on ORAC values of pearl millet since the values obtained (ranging from 6530.00 µmol TE/100 g to 7524.00 µmol TE/100 g) were approximately comparable to 8127.88 µmol TE/100 g for RPMF. The slight fluctuations in ORAC values during malting also correlated with TPC values as observed with fermentation and corroborated by Zhang *et al.* (2017:6) and Sindhu & Radhai (2018:138). This may be due to the proportional dynamics of bound and soluble phenolics concentrated in the testa and pericarp of the grain as these structures are ruptured during sprouting. The final ORAC value of malted pearl millet was lower, although not significantly ($p \leq 0.05$) when compared to RMPF.

ORAC measures the antioxidant scavenging activity of samples against peroxyl radicals generated by 2,2'-Azobis (2-methylpropionamidine) dihydrochloride (APPH). The scavenging efficacy of peroxyl radicals in pearl millet may indicate its potential to be utilised in the diet as a source of antioxidants key to managing disease conditions such as cancer and cardiovascular diseases, in which reactive oxygen species are causative factors (Chandrasekara & Shahidi, 2011:435).

3.3.7 Effect of fermentation and malting time on ferric reducing antioxidant power (FRAP) of pearl millet

Table 3.9 shows the FRAP values of pearl millet as influenced by fermentation and malting.

Table 3.9 Effect of fermentation and malting time on the ferric reducing antioxidant power (FRAP**)** of pearl millet flour[1]

Time (h)	**FRAP[2] (µmol AAE/100 g)**	
	Fermented pearl millet	Malted pearl millet
0	1266.47 ± 49.79[a]	1266.47 ± 49.79[a]
6	1391.48 ± 15.31[b]	ND
12	1311.12 ± 27.06[a]	ND
18	1318.77 ± 5.42[a]	1115.95 ± 10.82[b]
24	1289.43 ± 8.84[a]	989.67 ± 16.24[c]
30	1302.19 ± 26.88[a]	1136.36 ± 3.61[b]
36	1161.87 ± 46.56[b]	1498.63 ± 20.25[d]

[1]Results are reported as Mean ± Standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$, [2]Ferric reducing antioxidant power (AAE - Ascorbic acid equivalent). ND – Not done.

The FRAP of pearl millet ranged between 1161.87 to 1391.48 µmol AAE/100 g during fermentation. There was an initial significant ($p \leq 0.05$) increase in FRAP during the first 6 h yielding a peak value of 1391.48 µmol AAE/100 g. Thereafter, there was a significant ($p \leq 0.05$) drop to 1311.12 µmol AAE/100 g and a stable trend until 30 h. A final significant ($p \leq 0.05$) drop was noted at 36 h when FRAP reduced to 1161.87 µmol AAE/100 g, a value that is significantly ($p \leq 0.05$) less than the starting 1266.47 µmol AAE/100 g of RPMF. The FRAP trend during fermentation once again tremendously aligned with TPC content across almost all intervals.

FRAP values ranged between 983.29 and 1498.63 µmol AAE/100 g during malting. There was an initial significant ($p \leq 0.05$) drop from 1266.47 µmol AAE/100 g of RPMF to the lowest figure of 983.29 µmol AAE/100 g at 24 h. This was followed by a subsequent significant ($p \leq 0.05$) increase to 1129.56 µmol AAE/100 g and a resultant peak to 1498.63 µmol AAE/100 g at 30 and 36 h, respectively. The 15.5% increase in FRAP achieved in malting was comparable to Pushparaj & Urooj (2014: 63) who reported a 16.7% increase and somewhat contrary to the 10.2% decrease in two different pearl millet variants. Koren *et al.* (2019:3806) and Mahmoudi *et al.* (2015:537) also reported a resultant increase in FRAP during malting. Mahmoudi *et al.* (2015:537) attributed the resultant increase in TPC during malting to the kilning stage citing outcomes of friable tissues and improved extractability as potential reasons. This phenomenon was also noted in this study with a 36 h period yielding the optimum result for FRAP.

Ferric-reducing antioxidant power (FRAP) is used to measure the antioxidant power of sample extracts whereby the ferric-reducing ability is tested. Other than managing diseases, antioxidants in foods prevent deterioration in the flavour and nutritional quality of products (Pushparaj & Urooj, 2014:56). Elevated FRAP values in pearl millet flour are an indication of high antioxidant power and therefore a key aspect for improving its keeping quality.

3.4 Conclusion

Overall, fermentation and malting were noted to increase and decrease the viscosity of pearl millet flour, respectively. This result partially confirmed the third hypothesis, citing both processes to improve the pasting properties of pearl millet, as only malting led to a favourable outcome. Fermenting raw pearl millet flour increased its protein content significantly ($p \leq 0.05$) while malting instead, unexpectedly led to a slight decrease. Fermentation led to an unfavourable, significant decrease ($p \leq 0.05$), in bulk density whilst malting showed a fluctuating trend with the final value no different to that of RPMF. TPC fluctuated throughout both malting and fermentation periods of

pearl millet, owing to the release and degradation of bound phenolics by hydrolytic enzymes, with the overall trend indicating increased TPC for both processes. The antioxidant properties (ORAC and FRAP) also indicated a fluctuating trend through both fermentation and malting processes, mostly aligned with the observed TPC trend. However, at the end of both malting and fermentation processes, no significant changes were observed for ORAC, whilst FRAP significantly ($p \leq 0.05$) decreased and increased in FPMF and MPMF, respectively. The following chapter will explore the prospects of compositing malted and fermented pearl millet flours with Moringa leaf powder (MLP) to improve pearl millet's protein content and or minimise its saturated fat content by employing mixture design and numerical optimisation.

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CHAPTER FOUR

COMPOSITE OF FERMENTED AND MALTED PEARL MILLET FLOUR WITH *Moringa oleifera* LEAF POWDER FOR OPTIMUM PROTEIN USING MIXTURE DESIGN

Abstract

Pearl millet, one of the most widely consumed grains in the arid to semi-arid regions of Africa and India provides a substantial quantity of essential amino acids as well as fatty acids, minerals, and vitamins. However, it is deficient in the essential amino acid, lysine. To produce a more nutrient-balanced product, pearl millet has been combined with legumes. Legumes however present challenges such as anti-nutritional factors, poor digestibility, toxic components, and cause flatulence. Prospects of using *Moringa oleifera* leaf powder (MLP) to improve the protein content and overall nutritional quality of pearl millet were thus explored by this study. Malted pearl millet flour (MPMF) and fermented pearl millet flour (FPMF) were used in composite mixtures as the two processes were observed to improve the protein content and pasting properties of pearl millet, respectively. An I-optimal mixture design was used to determine the optimum composite flour whereby twelve mixtures with varying ratios of FPMF, MPMF ranging between 30–65% and MLP content ranging between 5–15% were generated. The mixtures were cooked, freeze-dried, and analysed for protein, fat content, and total phenolic content (TPC). Across the twelve mixtures the following ranges were acquired, protein (7.57–13.15 g/100 g), total fat (2.15–3.48 g/100 g), saturated fat (0.97–1.54 g/100 g), monounsaturated fat (0.60–1.11 g/100 g), poly-unsaturated fat (0.59–1.05 g/100 g) and TPC (115.48–789.74 mg/100 g). The data were fitted to a linear mixture model and the search for the optimum was done using Numerical Optimisation of Design-Expert version 10 for maximising protein and minimising saturated fatty acids. The linear model was suitable for explaining variation for total protein and saturated fat content with R^2 of 0.50 and 0.51, respectively. Increasing MLP increased protein content. Two final formulations were generated through the optimisation process: (1) 15:30:55 MLP, MPMF and FPMF respectively, with 12.41% protein and 1.49% saturated fat projected for maximising protein with the desirability of 0.865 and (2) 15:55:30 MLP, MPMF and FPMF respectively, with 11.84% protein and 1.25% saturated fat projected for maximising protein and minimising saturated fat with the desirability of 0.625.

4.1 Introduction

Pearl millet *(Pennisetum glaucum)* is widely cultivated in arid and semi-arid regions in India, that are characterised by low rainfall and infertile soils, on which other major cereals fail to yield significant harvests (Manga & Kumar, 2011:200). It provides protein with a substantial quantity of essential amino acids as well as fatty acids, minerals, vitamins and polyphenols (Issoufou *et al*., 2013:501). Pearl millet, however, lacks the essential amino acid lysine, which has been supplemented by compositing with protein-rich legumes (Issoufou *et al*., 2013:505).

Food compositing, referred to by other authors as blending, involves combining two different nutrient sources such as cereals and legumes to produce a more nutrient-balanced product that complementarily contains the benefits of the different combined individual foodstuffs (Nnam, 2001:251; Pelembe *et al*., 2002a:120; Anyango, 2009:29). In a study conducted by El-Fatah *et al.* (2013:1075), MLP yielded up to a 15.4% increase in crude protein content when composited at 7.5% with wheat, indicating its potential to improve protein content in cereal-based foods.

Mixture experiments are response surface experiments used in designing products whereby the product under investigation is made up of several components (Wangkananon *et al*., 2018:176). Statistical analysis and mixture design are utilised in synergy to evaluate the effect of combining two or more components and to forecast the response(s) for all probable formulations of the mixture. The utmost goal of mixture design is to find optimal proportions for each of the components that yield a highly desirable response for a selected variable, for example, a high protein content in this study (Giese *et al*., 2011:239; Goos *et al*., 2016:899; Wangkananon *et al*., 2018:176).

Extreme vertices design is a type of mixture design where lower and upper bound constraints are imposed on components to only cover a certain, smaller isolated portion within the simplex to sufficiently cover the desired design space. The main purpose of the extreme vertices design mixture is to explain the correlation between investigated variables and outcomes as it also can simultaneously optimise several variables to obtain the most favourable valid and objective response and conclusions, especially in areas that are not limited to food and beverages (Damiri *et al*., 2016:156; Zhang *et al*., 2015:5831).

This research sought to produce a composite of fermented and malted pearl millet flour with moringa oleifera leaf powder for optimum protein and or minimum saturated fat content using mixture design. Malting and fermentation were employed

based on observed benefits comprising improved pasting properties and increased protein content of raw pearl millet, respectively, in the present research.

4.2 Materials and Methods

4.2.1 Source of materials

Pearl millet grains were acquired from AGT Foods, Cape Town, South Africa whereas Moringa leaf powder (MLP) was purchased from SupaNutri Pty (Ltd), Graaff-Reinet Eastern Cape Province, South Africa. All chemicals were purchased from Merck (Pty) Ltd apart from sodium carbonate and gallic acid standard that were bought from Sigma-Aldrich (Pty) Ltd.

4.2.2 Production of malted and fermented pearl millet flours

Fermented pearl millet flour was produced as per section 3.2.2 employing a 36 h bulk fermentation period. Malted pearl millet flour was prepared as per section 3.2.3 employing a 36 h germination period.

4.2.3 Experimental Design

An I-optimal point exchange mixture design was used to estimate the effects of Moringa oleifera leaf powder (MLP), malted pearl millet flour (MPMF) and fermented pearl mill flour (FPMF) for high protein content composite flour. Each component was constrained with lower and upper limits of 5 to 15% for Moringa leaf powder, 30 to 65% for both malted pearl millet flour and fermented pearl millet flour for a 100 g batch (Table 4.1). Twelve mixtures with 4 interior points, 3 vertices, 2 third edge points and 3 centre edge points with varying ratios of MPMF, fermented pearl millet flour FPMF, and Moringa leaf powder MLP were generated (Table 4.2). The formulations were produced in randomised order, blended, and wet-cooked with the subsequent addition of MLP after brief cooling for about 10 mins.

Table 4.1 Mixture design constraints for composite pearl millet flour

Low limit		Constraint		High limit
5	≤	A: Moringa leaf powder	≤	15
30	≤	B: Malted pearl millet flour	≤	65
30	≤	C: Fermented pearl millet flour	≤	65
		A+B+C	=	**100**

Table 4.2 **Composite millet flour formulations from 3-component I-optimal point exchange mixture design**

Run	Component proportions		
	Moringa leaf powder (%)	Malted pearl millet flour (%)	Fermented pearl millet flour (%)
1	7.5	46.3	46.3
2	7.5	46.3	46.3
3	5.0	65.0	30.0
4	11.7	58.3	30.0
5	11.7	58.3	30.0
6	10.0	37.5	52.5
7	5.0	30.0	65.0
8	10.0	30.0	60.0
9	15.0	42.5	42.5
10	10.0	30.0	60.0
11	15.0	30.0	55.0
12	10.0	52.5	37.5

The wet cooking was carried out as follows: in a stainless-steel pot, the FPMF and MPMF were mixed in the specified ratios for 200 g batch sizes, combined with 200 g of water and then stirred to form a paste. In addition, 600 g of boiling water was added while continuously stirring gently. The resulting slurry was cooked on a hot plate stove, at medium heat with consistent stirring to reach 80–85°C and held for 10 minutes with periodic stirring. Another 200 to 400 g of water was added to the mixture during the initial stages of simmering to prevent excessive thickening of the mixture and achieve a consistent simmering throughout the cooking process. The mixture was then removed from the heat and allowed to cool to <50°C before the addition of a corresponding amount of MLP, and gently mixed with a spatula. The final paste was then transferred into stainless steel trays, frozen overnight and transferred into an ultra-freezer (SL 9002, Snijders Scientific, Holland) before freeze-drying using a freeze dryer (Genesis SQ Super XL-70, SP Scientific, USA). The dried samples were kept refrigerated at <5°C in sealed ziplocked plastic containers until analysed for protein, fat, and total phenolic content.

4.2.4 Protein content of mixtures

Protein content was determined as per section 3.2.7.

4.2.5 Fat Determination of mixtures

Total fat and fatty acid content were determined following the standard AOAC official method 996.06 (AOAC, 2005). Samples were taken through digestion, extraction and methylation processes followed by analysis using Agilent Technologies 7890B Gas Chromatograph (GC) fitted with Flame Ionised Detectors (FID). A 1 g portion of the sample was weighed into a 70 ml tube and the actual mass to a milligram was recorded. A 2 ml portion of internal standard solution and 2 ml ethanol were added to the sample and gently mixed with a vortex mixer. A 10 ml portion of hydrochloric acid (32%) was added to the mixture, with subsequent shaking. The test tubes were placed in a 70–80°C water bath with a shaker for 40 minutes, with intermittent gentle shaking every 10 minutes. Extraction was further done by adding 25 ml of diethyl ether, subsequent shaking for 5 minutes followed by the addition of 25 ml of petroleum ether and another 5 minutes of shaking. The mixture was allowed to separate into 2 layers, and the upper layer was carefully removed. The remaining layer was transferred into a 150 ml beaker and the ether was evaporated in a fume hood cupboard to dryness.

Derivatisation/methylation of the sample was done by reconstituting the residue in 3 ml chloroform and 3 ml diethyl ether with the subsequent transfer into a 10 ml tube and evaporating to dryness under a nitrogen gas stream. 2 ml of derivatising reagent (2% concentrated sulphuric acid in methanol) and 1 ml of toluene were added to the tube. The tubes were sealed tightly and placed in the oven at 100°C for 45 minutes. After cooling the tubes to room temperature, 5 ml water and 1 ml hexane were added with subsequent shaking for a minute. Layers were allowed to separate, and the top layer was carefully transferred to another tube. A pinch of anhydrous sodium sulphate was added to the solution with gentle shaking until clear. The final solution was transferred into a vial and placed in an autosampler tray for Gas chromatography analysis with the load method as fatty acid methyl esters (FAME). The sample weights recorded at the first stage were entered for corresponding samples before a sequence was set starting with a blank and initiating analysis.

4.2.6 Total phenolic content of mixtures

Total phenolic content was measured as per section 3.2.8.

4.2.7 Data Analysis

Multivariate analysis of variance (MANOVA) was used to determine the mean difference between treatments and compositions (at $p = 0.05$) while Duncan's

multiple ranges testes were employed to separate means where differences exist. To facilitate that, data or results were processed, trended, and analysed using the IBM Statistical Package for Social Science (IBM SPSS, version 26, 2019). The data from mixture design experiments was fitted to a linear mixture model and the search for the optimum was done using the Numerical Optimisation of Design Expert (Stat-Ease Design Expert, version 10, 2016).

4.3 Results and Discussion

Table 4.3 shows results from the analysis of the twelve mixtures, generated using the I-optimal point exchange mixture design, for protein, fat, and total phenolic content. Across the twelve mixtures the following ranges were acquired, protein (7.57–13.15 g/100 g), total fat (2.15–3.48 g/100 g), saturated fat (0.97–1.54 g/100 g), monounsaturated fat (0.60–1.11 g/100 g), poly-unsaturated fat (0.59–1.05 g/100 g) and TPC (115.48–789.74 mg/100 g).

Table 4.3 Protein, fat and fatty acids, and total phenolic content of mixtures[1]

Mixture	MLP (%)	MPMF (%)	FPMF (%)	Protein	Total Fat	SFA	MUFA	PUFA	TPC
R1	7.5	46.3	46.3	8.95 ± 0.36^{a,e}	2.63 ± 0.07^{a}	1.07 ± 0.03^{a}	0.75 ± 0.02^{a,c}	0.81 ± 0.02^{a}	128.86 ± 6.37^{a,c}
R2	7.5	46.3	46.3	9.87 ± 0.23^{b}	2.39 ± 0.07^{b}	1.12 ± 0.25^{a}	0.67 ± 0.03^{a,b}	0.61 ± 0.01^{b}	607.69 ± 47.00^{b}
R3	5.0	65.0	30.0	7.97± 0.77$^{c.d}$	2.95 ± 0.03^{c}	1.14 ± 0.12^{a}	0.83 ± 0.01^{a,c,d}	0.95 ± 0.05^{c}	128.71 ± 5.60^{a,c}
R4	11.7	58.3	30.0	8.38 ± 0.19^{a,c,d}	3.23 ± 0.18^{d}	1.27 ± 0.07^{b}	0.90 ± 0.06^{c,d,f}	1.05 ± 0.06^{d}	143.60 ± 8.76^{a,c}
R5	11.7	58.3	30.0	9.75 ± 0.34^{b,e}	2.15 ± 0.11^{e}	0.97 ± 0.04^{c}	0.60 ± 0.04^{b}	0.59 ± 0.04^{b}	789.74 ± 7.70^{e}
R6	10.0	37.5	52.5	8.58 ± 0.33^{a,d}	3.38 ± 0.18^{d,f}	1.39 ± 0.06^{d,e}	1.11 ± 0.28^{e}	0.99 ± 0.05^{c,d}	148.08 ± 13.07^{a,c}
R7	5.0	30.0	65.0	7.57 ± 0.33^{c}	3.48 ± 0.10^{f}	1.42 ± 0.04^{e}	1.02 ± 0.03^{e,f}	1.03 ± 0.03^{d}	115.48 ± 25.19^{a}
R8	10.0	30.0	60.0	10.57 ± 0.33^{b,f}	3.48 ± 0.01^{f}	1.44 ± 0.01^{e}	1.01 ± 0.01^{e,f}	1.03 ± 0.00^{d}	154.92 ± 8.00^{c,d}
R9	15.0	42.5	42.5	12.12 ± 0.39^{g}	2.84 ± 0.10^{c}	1.32 ± 0.06^{b,d}	0.81 ± 0.03^{a,c,d}	0.71 ± 0.17^{e}	194.60 ± 4.84^{f,g}
R10	10.0	30.0	60.0	11.07 ± 0.50^{f}	3.23 ± 0.04^{d}	1.47 ± 0.02^{e,f}	0.94 ± 0.02^{d,f}	0.81 ± 0.11^{a}	167.75 ± 15.86^{d,f}
R11	15.0	30.0	55.0	12.94 ± 1.19^{g,h}	3.29 ± 0.01^{d,f}	1.54 ± 0.00^{f}	0.95 ± 0.01^{d,f}	0.81 ± 0.00^{a}	211.35 ± 16.10^{g}
R12	10.0	52.5	37.5	13.15 ± 1.91^{h}	3.29 ± 0.16$^{d.f}$	1.47 ± 0.07^{e,f}	0.94 ± 0.04^{d,f}	0.87 ± 0.05^{a}	173.53 ± 6.30^{d,f}

[1]Results are reported as Mean ± Standard deviation. Means with different superscripts in each column are significantly ($p < 0.05$) different from each other. MLP – Moringa leaf powder, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour. SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, and TPC – total phenolic content.

4.3.1 Evaluation of Model Fit and Adequacy

Table 4.4 shows a summary of the model statistics and regression coefficients with key variables of the mixture experiment. The listed variables were fitted to a linear model, and the residual errors were calculated to determine the goodness of model fit.

Table 4.4 Regression coefficients and model summary statistics of the linear model for protein and fat content of optimisation mixtures

Response variables	**Regression (R^2)**	**Adjusted Regression (R^2)**	**Adequate precision**	***F*-value**	**P-value**
Protein	0.5011	0.3902	6.273	4.52	0.0438
Total Fat	0.2913	0.1338	3.253	1.85	0.2124
Saturated Fat	0.5103	0.4015	6.173	4.69	0.0402
MUFA[1]	0.3409	0.1944	3.648	2.33	0.1532

[1]MUFA – monounsaturated fatty acid.

The model values (F = 4.52; P = 0.0438) imply that the linear model was significant in describing component effects (MPMF, FPMF and MLP) on the protein content of the mixtures. There was only a 4.38% chance that the high *F*-value could occur due to noise (unexplained variability) in data, a further indication that model terms were significant. Moreover, the model lack of fit (F = 6.31; P = 0.0795) was not significant, a factor demonstrating sufficient model goodness-of-fit. The predicted regression (0.2340) was reasonable and in agreement with the adjusted regression (0.3902) with a less than 0.2 difference. A predicted regression value within the 0.2 range of the adjusted regression value signifies a reasonable agreement of the linear model. The adequate precision (a measure of signal-to-noise ratio) value of 6.273, greater than 4, was desirable and therefore indicated that the model could be used to navigate the design space.

In explaining component effects on the total fat content of the mixtures, the model values (F = 1.85; P = 0.2124) indicated that the linear model was not significant relative to the noise. There was also a high chance (21,24%) that the large *F*-value occurred due to unexplained variability, a phenomenon that further discredits model adequacy. Moreover, the low (less than 4) adequate precision value of 3.253 indicated an inadequate signal-to-noise ratio ultimately indicating that the model could not be used to navigate the design space.

The component effects on the saturated fat content of the mixtures were adequately described by the linear model with model values (F = 4.69; P = 0.0402). There was only a 4.02% chance that the high *F*-value could occur due to noise, a

further indication that model terms were significant. The model lack of fit (F = 1.51; P = 0.3941) was not significant, indicating adequate model goodness of fit. The predicted regression (0.2222) was reasonable and in agreement with the adjusted regression (0.4015) with a less than 0.2 difference. The adequate precision value of 6.173, was desirable and therefore indicated that the model could be used to navigate the design space.

Lastly, model values (F = 2.33; P = 0.1532) indicated that the linear model was not significant in explaining the component effects on the mono-unsaturated fat content of mixtures. There was a high chance (15,32%) that the large F-value occurred due to noise, an indication of model inadequacy. Moreover, the low (<4) adequate precision value of 3.648 indicated an inadequate signal-to-noise ratio, meaning that the model could not be used to navigate the design space.

Based on information from Table 4.4 and deductions thereof, the linear model was found appropriate to determine the effect of components (MPMF, FPMF and MLP) on the protein and saturated fat content of the flour mixtures. Since one of the assumptions used in the statistical model of this experiment was that the experimental errors or residuals were normally distributed, the Normality Plot of Residuals and the Box-Cox Plot diagnostic tools were used to confirm the normality of data. All four Normality Plot of Residuals (not shown) demonstrated that residuals fell along a straight line, conjecturing that the residuals followed a normal distribution and that a change in transformation would not improve the analysis. If the plot of residuals had shown a random scatter and major deviations from the straight line, transformation of the response would have been essential as this would have been an indication that the errors are independent (Krishnaiah & Shahabudeen, 2012:32; Jeirani *et al*., 2012:4). Furthermore, the Box-Cox diagnostic tool which works on a principle of comparing the natural log of the sum of the squares of errors or residuals versus lambda also indicated no need for transformations to the response variables paving the way for model graphs to be generated (Jeirani *et al*., 2012: 4).

4.3.2 Effects of mixture components on the protein content of the composite flours

Figure 4.1 shows the trace (Piepel) plot and response surface plot for the effect of mixture components (A: MLP, B: MPMF and C: FMPF) on the protein content of composite flour mixtures. The trace (Piepel) plot, Figure 4.1(A) indicates the protein content trend as MLP, MPMF and FPMF vary both positively and negatively (increase and decrease) from a reference blend.

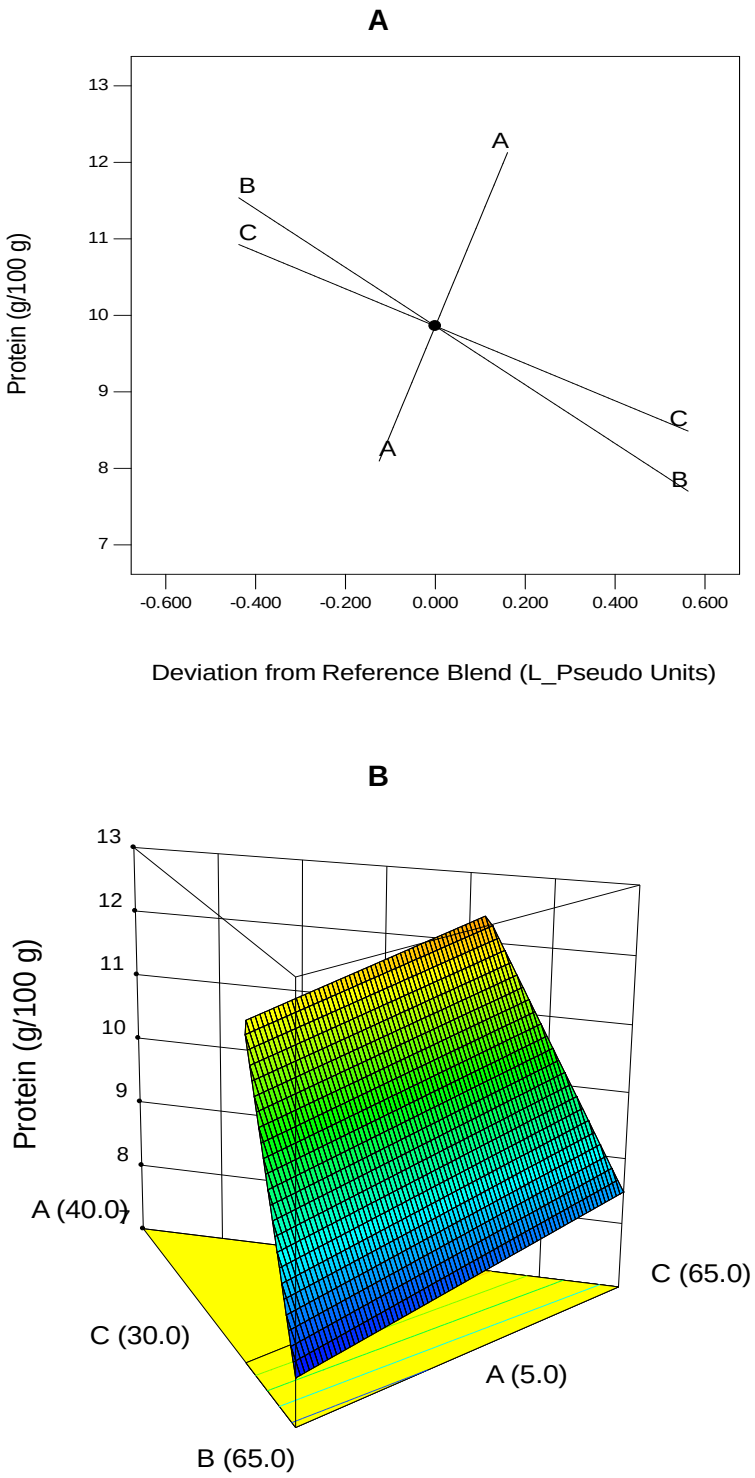

Figure 4.1 (A) Trace (Piepel) plot and (B) response surface plot for the effect of 3 components (A: MLP, B:MPMF and C:FMPF) on the protein content of composite flour mixtures.

The results in this figure show that a positive deviation from the reference blend (increase) of MLP (A) directly yielded a notable increase in the protein content of the

mixture. Conversely, an increase in MPMF (B) and FPMF (C) led to a decrease in protein content although it was observed that increasing MPMF reduced protein content to a greater extent than FPMF. Ultimately, the protein content was most sensitive to variation in MLP content. The 3D response surface plot Figure 4.1(B) shows that the highest protein content was found at a point within the design space where MLP and FPMF were both at the highest possible proportions. The lowest protein content was found in the MPMF vertex (representing the peak MPMF) whilst the FPMF vertex indicated a slightly elevated protein content. The protein content trends observed in the design space were in line with trends observed in Table 4.3, where higher protein content was observed in mixtures with elevated MLP content (10–15%). Moreover, formulations R3 and R7 with the lowest MLP content also showed the lowest protein content (<8.00 g/100 g). This phenomenon indicates the high potential of MLP as a protein enrichment component in pearl millet flour or any other cereal for that matter.

4.3.3 Effects of mixture components on the saturated fat of the composite flours

Figure 4.2 shows the trace (Piepel) plot and response surface plot for the effect of mixture components (A: MLP, B: MPMF and C: FMPF) on the saturated fat content of composite flour mixtures. The trace (Piepel) plot, Figure 4.2(A) indicates the saturated fat content trend as MLP, MPMF and FPMF vary both positively and negatively from a reference blend. The results in this figure show that a positive deviation (increase) of components A and C (MLP and FPMF, respectively) from the reference blend translated into a projected increase in saturated fat content.

Conversely, a positive deviation (increase) in component B (MPMF), led to a decrease in the saturated fat content. Overall, the saturated fat content was more sensitive to changes in the MLP and MPMF, with the latter showing a greater impact. The 3D response surface plot Figure 4.2(B) shows that the highest saturated fat content was found at a point within the design space where MLP was at the highest possible proportion coupled with an equally high FPMF content. The lowest saturated fat content was found in the MPMF vertex (peak MPMF content) whilst the FPMF vertex (peak FPMF content) indicated a significantly elevated saturated fat content. The saturated fat content trends observed in the design space aligned with trends observed in Table 4.3, where peak saturated fat content was observed in mixture R11 with maximum MLP (15%), high FMPF (55%) and minimum MPMF (30%) proportions.

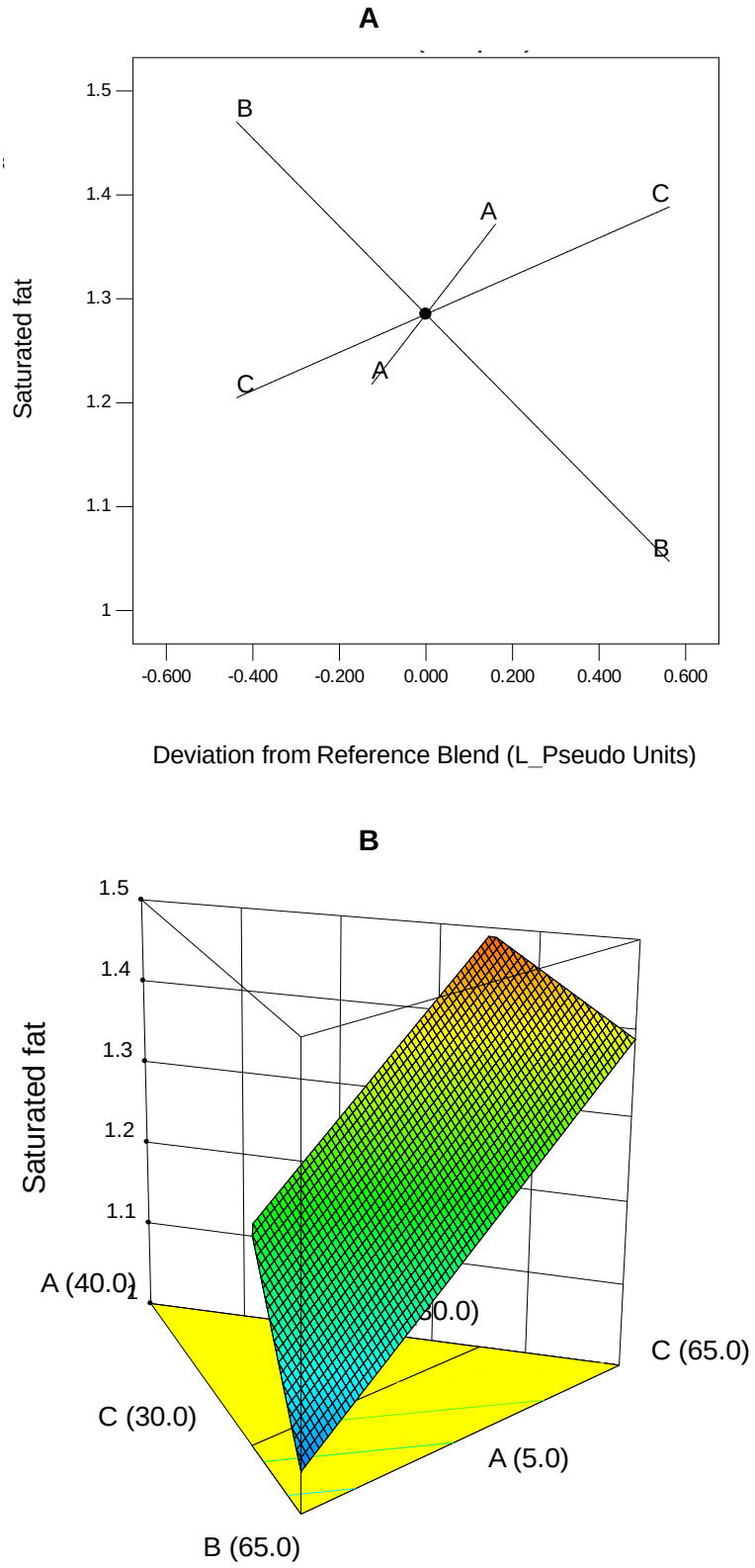

Figure 4.2 (A) Trace (Piepel) plot and (B) response surface plot for the effect of 3 components (A: MLP, B:MPMF and C:FMPF) on the saturated fat content of composite flour mixtures.

Ultimately, with saturated fat being long associated with a high risk of coronary heart diseases, it would appear ideal to employ a mixture with a maximum possible proportion of MPMF and essentially minimum FPMF, to minimise the saturated fat content of the composite flour mixture.

4.3.4 Numerical optimisation of protein and saturated fat content

Based on the data obtained from Table 4.4, whereby both protein and saturated fat content responses indicated high regression (≥0.50), the two responses were employed to be optimised by the components (MLP, MPMF and MPMF).

The first goal for optimisation of each component was specified as follows: component A (MLP) range 5–15%, component B (MPMF) range 30–65% and component C (FPMF) range 30–65%, while maximising protein content. Table 4.5 shows the numerical optimisation solution for maximising protein content.

Table 4.5 Optimisation Solution 1 (maximum protein)

Sol.	MLP	MPMF	FPMF	**Nutritional Parameters (g/100 g)**						Desirability
	(%)	(%)	(%)	Protein	T.Fat	SFA	TPC	PUFA	MUFA	
1	15.0	30.0	55.0	12.41	3.23	1.49	0.00	0.00	0.95	.867

Sol. Solution MLP – Moringa leaf powder, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, T.Fat – Total Fat, SFA – Saturated fatty acids, TPC – Total phenolic content, PUFA – Polyunsaturated fatty acids, MUFA – Monounsaturated fatty acids.

The best optimal solution with a desirability of 0.867 was found at 15:30:55 (w/w/w) proportions of MLP, MPMF and FPMF flours, respectively, yielding a predicted response of 12.41 g/100 g for protein content.

The second goal for optimisation of each component was specified as follows: component A (MLP) range 5–15%, component B (MPMF) range 30–65% and component C (FPMF) range 30–65%, while maximising protein content and minimising saturated fat content. Table 4.6 shows the numerical optimisation solutions for maximising protein content while minimising saturated fat content.

Table 4.6 Optimisation Solution 2 (maximum protein and minimum saturated fat)

Sol.	MLP (%)	MPMF (%)	FPMF (%)	**Nutritional Parameters (g/100 g)**						Desirability
				Protein	T.Fat	SFA	TPC	PUFA	MUFA	
1	15.0	55.0	30.0	11.85	2.75	1.25	0.77	0.86	249.19	.625
2	12.8	57.2	30.0	10.93	2.74	1.21	0.77	0.86	249.19	.595

Sol. Solution, MLP – Moringa leaf powder, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour. T. Fat – Total fat, SFA – Saturated fatty acids, TPC – Total phenolic content, PUFA – Polyunsaturated fatty acids, MUFA – Monounsaturated fatty acids.

Optimal solution 1 in Table 4.6 was selected, with a desirability of 0.625 found at 15:55:30 (w/w/w) proportions of MLP, MPMF and FPMF flours, respectively. The predicted responses under these conditions were 11.85 g/100 g for protein content and 1.25 g/100 g for saturated fat content.

Overall, it was evident that a favourable response for maximising the protein content of composite flours would be achieved under conditions where both MLP and FPMF were at the maximum possible proportions. A dual effort to maximise protein content while minimising saturated fat content however required a shift on experimental conditions calling for a maximum possible proportion of MPMF and conversely, minimum FPMF proportion while MLP remained at the maximum (15%) proportion.

4.5 Conclusion

The linear mixture model was found to be suited to determine the effects of the three components (MLP, MPMF and FPMF) on the protein and saturated fat content of composite flour mixtures. MLP was noted to contribute to more protein in the mixtures as peak protein was achieved at peak MLP content. Saturated fat was found to be most sensitive to changes in MPMF content in the composite flour mixtures, with a maximum possible proportion of MPMF (55%) favoured for minimising saturated fat content. Two solutions were acquired through the numerical optimisation process, optimisation solution 1 (OS1) with 15:30:55 MLP, MPMF and FPMF (w/w/w) ratios respectively, where the goal was to maximise protein content projected at 12.41% and the desirability of value 0.865. Optimisation solution 2 (OS2), with goals of maximising protein, whilst minimising saturated fat had ratios of 15:55:30 MLP, MPMF and FPMF (w/w/w) respectively with protein and saturated fat projected at 11.84% and 1.25%, respectively, and the desirability value of 0.625. It was therefore deduced that a maximum proportion of MLP (15%) would be ideal to achieve maximum protein content in composite food products containing MLP,

MPMF and FPMF. The subsequent chapter of this study delves into the evaluation of the model's accuracy. In this process, the proposed optimum compositions of flour mixtures (OS1 and OS2) will be produced and analysed for the applicable responses, that is, protein and saturated fat content. Additionally, both mixtures will be analysed for proximate composition and, biochemical and physicochemical properties.

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CHAPTER FIVE

NUTRITIONAL, PHYSICOCHEMICAL AND ANTIOXIDANT PROFILE OF FERMENTED, MALTED PEARL MILLET AND MORINGA LEAF COMPOSITE INSTANT FLOURS

Abstract

Two final formulations of malted pearl millet flour (MPMF), fermented pearl millet flour (FPMF) and shade-dried moringa leaf powder (MLP) generated through the optimisation process, (OS1) 15:30:55 MLP, MPMF and FPMF and (OS2) 15:55:30 MLP, MPMF and FPMF respectively, were cooked and freeze-dried. The final formulations (OS1 and OS2) were analysed for nutritional, biochemical, and physicochemical properties and the results were as follows. Protein content ranged between 12.60–13.51 g/100 g with lysine content ranging between 0.45–0.55 g/100 g. Total fat content ranged between 3.90–6.59 g/100 g whilst saturated, monounsaturated, and poly-unsaturated fats ranged between 1.54–2.43 g/100 g, 1.03–1.69 g/100 g, 1.32–2.77 g/100 g respectively. Ash content was at 2.93 % for both formulations. Vitamin C content ranged between 33.0 to 49.3 mg/100 g whilst vitamin A was not detected. Polyphenol content and antioxidant properties were in the following ranges, total phenolic content (477.44–513.33 mg GAE/100 g), FRAP (1587.92–1677.21 µmol AAE/100 g) and ORAC (11049–11700 µmol TE/100 g) while tannins were not detected across all samples. Bulk density ranged between 32.31–33.21 $kg.m^{-3}$. Final viscosity and peak viscosity ranged between 46.33–63.67 Cp and 27.67–45.67 Cp, while no pasting was achieved. Water absorption index, water holding capacity and water solubility index ranged between 419.0–482.0%, 3.17–3.40 $g.g^{-1}$ and 21.8–33.0%, respectively. Water activity and moisture content ranged between 0.327–0.330 and 4.15–4.50%. Compositing pearl millet with MLP increased the protein and lysine content by up to 22% and improved the protein composition overall. The addition of MLP also improved the mineral content of pearl millet, with an ash content increase of 75% observed in both OS1 and OS2.

5.1 Introduction

Pearl millet flour is mostly used to make porridge or beverages, for millions of people mainly in the underdeveloped regions of Sub-Saharan Africa, India, and China (Issoufou *et al*., 2013:501). As a cereal, it is individually or as a composite with other cereals and or legumes, used as a nutrient source for weaning children in developing countries. Ready-to-eat (RTE) pearl millet foods have also been made available by employing extrusion cooking (Taylor *et al*., 2010:18; Obilana, 2013:39).

In Namibia, Southern Africa, pearl millet is composited with sorghum in the production of a traditional millet beverage called *oshikundu* (Taylor *et al*., 2010:18). In the Sahel region, Northern Africa, sorghum, and pearl millet couscous is a traditional staple food with Senegal producing it commercially. Commercial processing employs the decortication of grains, with subsequent milling and repetitive agglomerating, steaming and sifting of the flour to produce a uniform particle size (Taylor *et al*., 2010:17). In pearl millet and cowpea composites, the flours are mixed with water in ratios up to 1:4 (s/w) and cooked or heated with continuous stirring before press drying and milling. The flours are then reconstituted with warm water, and sugar is added if preferred to produce either a paste or liquid beverage (Almeida-Dominguez *et al*., 1993:215). Fermented, and germinated cereal flours have also been used to produce instant pearl millet foods such as *fura,* a West African thick porridge (Inyang & Zakari, 2008a:9)

In general, numerous composite foods have been developed, with pearl millet and other cereals utilising legumes (cowpea, chickpea, and pulses) and have produced nutrient improvement with minimal reduction in acceptability of the final food product (Pelembe *et al*., 2002a:125; Anyango, 2009:94). Compositing cereals with moringa leaf powder at low proportions yielded notable increases in protein and ash content of cereal food with comparable acceptability to control samples (El-Fatah *et al*., 2013:1075; Nour & Ibrahim, 2016:673). However, there has not been much research on the use of moringa leaf powder to improve the nutrient content of pearl millet.

Experimental outcomes from the previous chapter indicated successes in the application of the linear mixture model to determine the effects of moringa leaf powder (MLP), malted pearl millet flour (MPMF), and fermented pearl millet flour (FPMF) on the protein and saturated fat content of composite flour mixtures. The numerical optimisation process yielded two solutions of mixtures, optimisation solution 1 (OS1) with 15:30:55 MLP, MPMF and FPMF (w/w/w) ratios respectively, where the goal was to maximise protein content projected at 12.41%. Optimisation solution 2 (OS2), with goals of maximising protein, whilst minimising saturated fat

had ratios of 15:55:30 MLP, MPMF and FPMF (w/w/w) respectively with protein and saturated fat projected at 11.84% and 1.25%, respectively.

This chapter covers the preparation of fermented, malted pearl millet and moringa leaf instant flours as per mixture ratios generated through the numerical optimisation process from Chapter four. The main objective of the chapter was to examine the nutritional, physicochemical and antioxidant profiles of malted, fermented pearl millet and moringa leaf composite instant flours. In conducting the objective, the chapter further evaluated the accuracy of the model applied in chapter four, particularly through the analysis of protein and saturated fat content results in comparison with model projections.

5.2 Materials and Methods

5.2.1 Source of materials and equipment

Pearl millet grains were acquired from AGT Foods, Cape Town, South Africa. Moringa leaf powder was purchased from SupaNutri Pty (Ltd), Graaff-Reinet Eastern Cape Province, South Africa. All chemicals were purchased from Merck (Pty) Ltd apart from Sodium Carbonate and Gallic acid standard there were bought from Sigma-Aldrich (Pty) Ltd. The Ultra Performance Liquid Chromatography (UPLC) and relevant reagents were accessed at the University of Stellenbosch, South Africa. The Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was accessed at the University of Western Cape. The rest of the equipment used for the preparation of composite instant flours and reagents and conducting analysis were accessed at the Cape Peninsula University of Technology, South Africa.

5.2.2 Blending of malted and fermented pearl millet flour

Malted pearl millet flour (MPMF) and fermented pearl millet flour (FPMF), produced as per Chapter 3 (for 36 h respective periods), were weighed into 1000 g batches as per mixture ratios generated from the optimisation process solutions OS1 (15:30:55) and OS2 (15:55:30) MLP, MPMF and FPMF respectively. For OS1, 150 g, 300 g and 550 g of MLP, MPMF and FPMF, respectively, were weighed and set aside. For OS2, 150 g, 550 g and 300 g of MLP, MPMF and FPMF, respectively, were weighed and also set aside. The respective weighed portions of MPMF and FPMF (for OS1 and OS2) were subsequently blended with a spatula until sufficiently mixed.

5.2.3 Wet cooking of blends, the addition of MLP and drying of fermented, malted pearl millet and moringa leaf instant flours

The blended FPMF and MPMF portions (for OS1 and OS2) were combined with 850 ml portions of cold water, in 5 L stainless-steel pots. The cold slurries were stirred gently, and 2550 ml and 1700 ml portions of boiling water were added to OS1 and OS2 slurries, respectively. The warm slurries were cooked on a hot plate stove, at medium heat with consistent stirring to reach 80–85°C and held for 12 mins with periodic stirring. The mixtures were then removed from the heat and allowed to cool to < 50°C before the addition of corresponding amounts of MLP, and gently mixed with a spatula to facilitate the uniform distribution of all components. The cooked pastes were then transferred into stainless steel trays, frozen overnight and transferred into an ultra-freezer (SL 9002, Snijders Scientific, Holland) before being freeze-dried using a freeze dryer (Genesis SQ Super XL-70, SP Scientific, USA). The dried samples were milled using a universal cutting mill with a 0.75 mm sieve (Pulverisette 19, Fritsch, Germany) and kept refrigerated in sealed ziplock plastic containers until the time of analysis.

Figure 5.1 shows the processing flow diagram to produce the fermented, malted pearl millet and moringa leaf composite instant flours from the weighing of starting components to the packaging of the finished product.

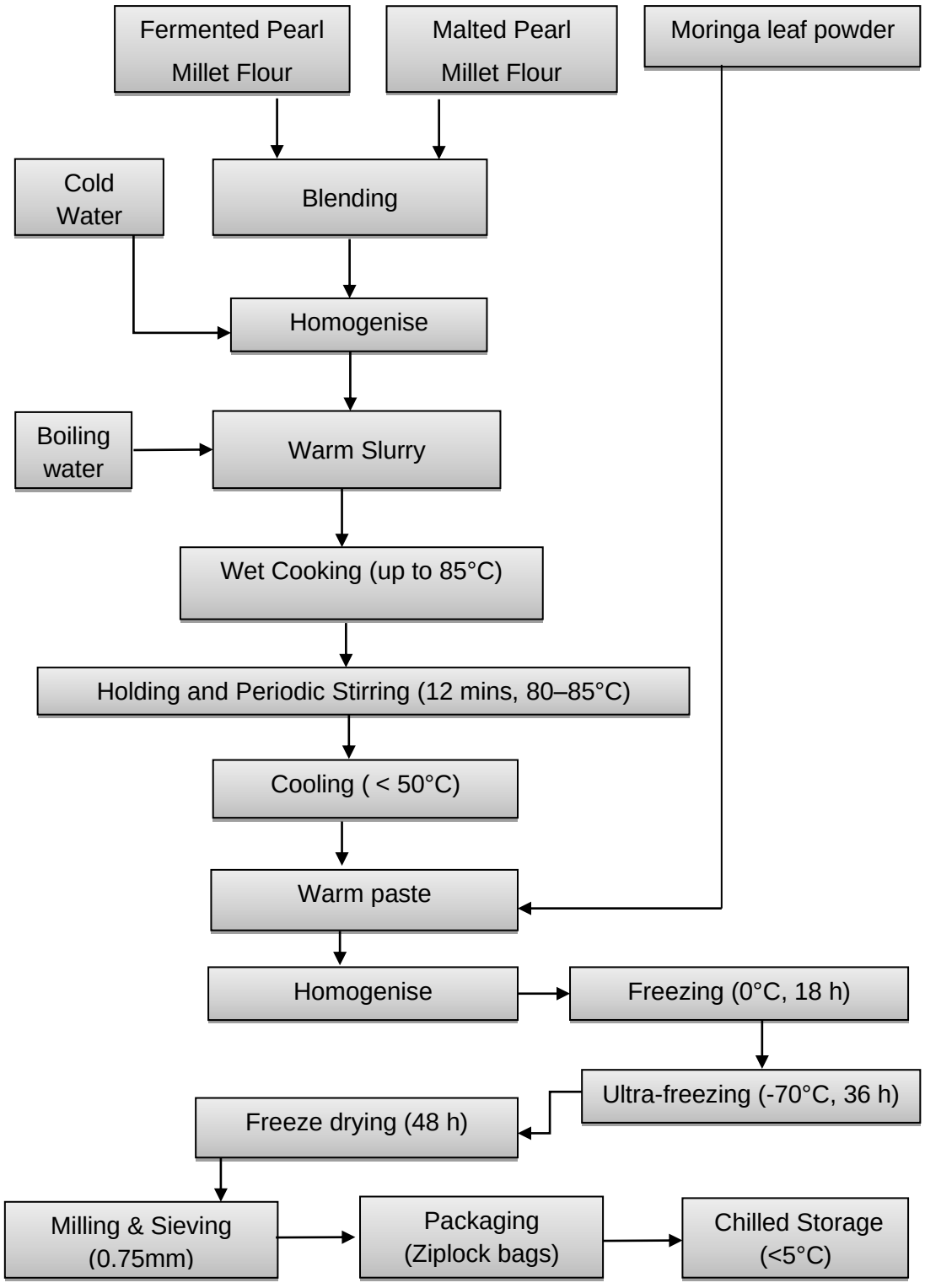

Figure 5.1 Flow diagram for the production of fermented and malted pearl millet and moringa leaf composite instant flours.

5.2.4 Nutritional properties of fermented, malted pearl millet and moringa leaf composite instant flours

The fermented, malted pearl millet and moringa leaf composite instant flours were analysed for protein and amino acid content, fats and fatty acids, sugars, vitamins A and C, ash content and minerals. All tests were done in triplicate except for amino acids, sugars, and vitamins A and C which were done in duplicate.

5.2.4.1 Determination of protein content and amino acid analysis of fermented, malted pearl millet and moringa leaf composite instant flours

The protein content of fermented, malted pearl millet and moringa leaf composite instant flours was determined as per section 3.2.7. The amino acid analysis of pearl millet and moringa composite instant flours was conducted based on a procedure outlined by Fiechter and Mayer (2011:1354) with minor modifications. The flour samples were derivatised and then analysed using ultra-performance liquid chromatography (UPLC) with UV detection. The following materials were used. The AccQ-Tag Ultra amino acid kit with derivatisation kit (Waters Corporation, USA), AccQ-Tag Ultra C18 2.1 x 100 mm x 1.7 µm column, Eluents A and B for use on the Waters Acquity UPLC system with a photodiode array (PDA) detector. The derivatisation kit contained, AccQ-Tag derivatising agent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)), dry acetonitrile for preparing the AQC, and a sodium borate buffer.

Eluent A (50 ml) was mixed with 950 ml of deionised water and eluent B was used as supplied. Weak wash and strong wash solvents were prepared to make 5% acetonitrile water and 95% acetonitrile in water, respectively. The derivatisation agent (AQC) was prepared as follows: 1 ml of dry acetonitrile (in a sealed vial from a kit) was added to the Reagent A vial containing 3 mg of AQC. This vial was then heated, vortexed and sonicated to ensure complete reagent dissolution. A 200 ppm internal standard (IS) of L-Norvaline was prepared by diluting it with MilliQ water.

Hydrolysis of proteins in the flour samples was done by weighing 100 mg of the sample into a 4 ml screw cap HPLC vial and adding 0.5 ml of 6N HCl, and subsequent full submersion in the HCl was done employing vortexing. The vials were flushed with Nitrogen gas to eliminate oxygen before closing the lids to prevent oxidative degradation of labile amino acids and subsequently placed in a preheated oven at 110°C overnight for 18 hours and allowed to cool. The hydrolysate was then filtered using centrifuge filters (Corning® Costar® Spin-X tubes) and the filtrate was transferred to Eppendorf tubes and dried down using a speed vac before reconstituting in a borate buffer. Sodium hydroxide (6M) was used to neutralise the

hydrolysed samples using an initial 5x (200 µl sample + 200 µl NaOH + 600 µl H2O) dilution, followed by a 10x (100 µl sample + 700 µl H2O + 200 µl IS) as protein content was estimated to be between 7–20%.

Derivatisation was conducted by transferring 70 µl of borate buffer into a 200 µl glass insert in a 2 ml glass vial. A 10 µl diluted sample/standard solution was added and followed by 20 µl AQC reagent before capping vials and vortexing to adequately mix. The vials were then transferred to an oven and heated at 55°C for 10 minutes before being loaded onto an autosampler tray. Amino acid separation and detection were performed using a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) (Waters Corporation, USA) fitted with a photodiode array (PDA) detector. Instrument control and data acquisition were performed using MassLynx software which integrates the peaks at the defined retention times and plots calibration curves for each amino acid based on the peak response (peak area/internal standard peak area) against concentration.

5.2.4.2 Determination of total sugar content of fermented, malted pearl millet and moringa leaf composite instant flours

Total sugar analysis of fermented, malted pearl millet and moringa leaf composite instant flours was conducted based on the AOAC 982.14 (AOAC, 2005) with minor changes. Ethanol was used for sugar extraction with the aid of heat and agitation before filtration, and the extract was analysed with a High-Performance Liquid Chromatograph fitted with a refractive index detector (HPLC-RID).

5.2.4.3 Determination of in-vitro starch digestibility of fermented, malted pearl millet and moringa leaf composite instant flours

In vitro starch digestibility of the fermented, malted pearl millet and moringa leaf composite instant flours was determined using a method described by Onyango *et al.* (2004:829). A 50 mg flour sample was dissolved in 1 ml 0.2 M phosphate buffer (pH 6.9). Porcine pancreatic amylase (20 mg) was dissolved in 50 ml of the same buffer and 0.5 ml added to the sample suspension and incubated at 37°C for 2 h. A 1 ml portion of 3,5,-dinitrosalicyclic acid was immediately added and the mixture was heated for 10 min in a boiling water bath. After cooling, the solution was made up to 25 ml with distilled water and filtered using Whatman No. 1 filter paper before measurement of absorbance in a 5 mm cuvette at 510 nm using an Ultrospec 1000 (Pharmacia Biotech, Cambridge, England). A blank for each sample was prepared by incubating the sample first and 3,5,-dinitrosalicyclic acid was added before the addition of the enzyme solution. A standard curve was prepared using solutions

containing known concentrations of maltose monohydrate (0, 10, 30, 50, 70 mg/l). Microsoft Excel was used to plot the standard curve and to calculate the concentration of starch digestion products in test solutions. The values were expressed as mg maltose/g starch.

5.2.4.4 Determination of fats and fatty acids content of fermented, malted pearl millet and moringa leaf composite instant flours

Fat and fatty acid determination of fermented, malted pearl millet and moringa leaf composite instant flours were done as per 4.2.5.

5.2.4.5 Determination of ash and mineral content of fermented, malted pearl millet and moringa leaf composite instant flours

The ash content of the fermented, malted pearl millet and moringa leaf composite instant flours was determined using the muffle furnace method, AOAC Method 923.03 (AOAC, 2005). The mineral content of fermented, malted pearl millet and moringa leaf composite instant flours was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Spectro Analytical Instruments GmbH, Germany). Ash, from approximately 4 g of the flour samples, was weighed into Erlenmeyer flasks containing 20 ml of concentrated nitric acid (HNO_3) and 1 ml of hydrogen peroxide (H_2O_2). The samples were then digested in an oven at 100°C for 45 minutes and cooled down to ambient temperature before being transferred into a volumetric flask and marked up to 100ml with Milli-Q water (Millipore, Bedford, MA). The samples were thereafter analysed using the inductively coupled plasma spectrometer (Spectro Analytical Instruments GmbH, Germany).

5.2.4.6 Determination of vitamin C content of fermented, malted pearl millet and moringa leaf composite instant flours

The vitamin C content of fermented, malted pearl millet and moringa leaf composite flours was determined as per the AOAC 984.26 method whereby sample extraction was done using 5% metaphosphoric acid with the aid of agitation followed by centrifugation and filtration (AOAC, 2005). The ascorbic acid content was determined using fluorometric detection with a High-Performance Liquid Chromatograph fitted with a UV detection unit.

5.2.5 Physicochemical properties of fermented, malted pearl millet and moringa leaf composite instant flours

All physicochemical properties analyses of fermented, malted pearl millet and moringa leaf composite instant flours were done in triplicates. Bulk density and pasting properties of fermented, malted pearl millet and moringa leaf composite instant flours were determined as per sections 3.2.6 and 3.2.5 respectively. The water absorption index (WAI) and water solubility index (WSI) of fermented, malted pearl millet and moringa leaf composite instant flours were measured according to a method outlined by Obilana *et al.* (2014:251) as adapted from Anderson *et al.* (1969:372). The WAI was measured by the dissolution of 2.5 g flour samples in 30 ml of warm (30°C) distilled water before centrifugation and subsequent weighing of the gel yielded after pouring supernatant into an evaporating dish. The WSI was determined from the weight of dried solids recovered by evaporating supernatant from the WAI test, where WSI was indicated by the weight of dissolved solids divided by the weight of dry solids (2.5 g flour sample) expressed as a percentage. Calculations for WAI and WSI were done as per Equation (5.1) and Equation (5.2) Obilana *et al.* (2014:92).

$$WAI\ (\%) = \frac{weight\ of\ sediments}{weight\ of\ dry\ solids} \text{ x } 100 \qquad \text{Equation (5.1)}$$

$$WSI\ (\%) = \frac{weight\ of\ solids\ dissolved\ in\ supernatant}{weight\ of\ dry\ solids} \text{ x } 100 \qquad \text{Equation (5.2)}$$

Water holding capacity (WHC) of fermented, malted pearl millet and moringa leaf composite instant flours was determined according to methods outlined by the AACC (2000) and Traynham *et al.* (2007:152) with slight modification. A 1 g portion of flour sample was weighed into a pre-weighed 50 ml test tube and shaken vigorously for approximately 2 minutes until the flour was thoroughly suspended. The hydrated flour sample was left to stand for 10 minutes and subsequently centrifuged at 3000 rpm for 15 minutes. The cleared supernatant was drained out and the residue in the tube was weighed and recorded. The WHC was then calculated as per Equation (5.3) adapted from (Traynham *et al*., 2007:152).

$$WHC\ (g/g) = \frac{[W3 - W1] - W2}{W1} \text{ x } 100 \qquad \text{Equation (5.3)}$$

Where: W_1 = Weight of test tube in grams, W_2 = Weight of sample in grams, W_3 = Weight of residue in the test tube in grams and WHC was expressed as grams of water held per gram of sample.

Water activity (A_w) of fermented, malted pearl millet and moringa leaf composite instant flours was measured using a water activity meter (Rotronic HC2-AW, Rotronic AG, Switzerland). Moisture content (MC) of fermented, malted pearl millet and moringa leaf composite instant flours was assayed using a procedure based on AOAC Method 934.01 Air Oven Method (AOAC, 2000). A crucible dish was dried at 105°C for 3 h and transferred to a desiccator to cool before weighing (W_0). A 3 g portion (W_1) of the flour sample was weighed into the empty crucible and spread flat before placing into the oven for drying at 105°C for 3 h. The dry flour sample was subsequently cooled in a desiccator and reweighed (W_2). Moisture content was calculated as per Equation (5.4) adapted from (AOAC, 2000).

$$MC\ (\%) = \frac{\mathrm{W1} - [\mathrm{W2} - \mathrm{W0}]}{\mathrm{W1}} \qquad \text{Equation (5.4)}$$

Where: W_0 = Weight of crucible in grams, W_1 = Weight of sample in grams and W_2 = Weight of dried sample in a crucible in grams.

5.2.6 Phenolic and antioxidant properties of fermented, malted pearl millet and moringa leaf composite instant flours

Total phenolic content (TPC), oxygen radical absorbance capacity assay (ORAC) and ferric reducing antioxidant power assay (FRAP) and tannins of fermented, malted pearl millet and moringa leaf composite instant flours were analysed as per sections 3.2.8, 3.2.9 and 3.2.10, respectively.

5.2.7 Data Analysis

Analysis of variance (ANOVA) was used to determine the mean difference between treatments at p = 0.05. Duncan's multiple range test was employed to separate means where differences exist using the IBM Statistical Package for Social Science (IBM SPSS, version 26, 2019).

5.3 Results and Discussion

5.3.1 Nutritional properties of fermented, malted pearl millet and moringa leaf composite instant flours

Table 5.1 shows the proximate composition of fermented, malted pearl millet and moringa leaf composite instant flours alongside, raw pearl millet and inputs of the composite flours.

Table 5.1 Sugar composition and starch digestibility of fermented, malted pearl millet and moringa leaf instant flours (g/100 g)[1]

	Proximate composition (%)				
	Moisture	Ash	Protein	Total Fat	Total Sugar
RPMF	9.47 ± 0.04[a]	1.67 ± 0.02[a]	11.12 ± 0.30[a]	5.28 ± 0.41[a]	1.10 ± 9.27[a]
FPMF	2.89 ± 0.04[e]	1.61± 0.04[a]	12.40± 0.33[b]	4.88± 0.22[a,d]	2.00 ± 4.80[e]
MPMF	7.87 ± 0.06[d]	1.59 ± 0.08[a]	9.98 ± 0.43[c]	4.33 ± 0.88[c,d]	3.50 ± 4.80[d]
MLP	7.11 ± 0.12[f]	10.76 ± 0.13[c]	26.32 ± 1.25[d]	2.71 ± 0.29[e]	4.70 ± 4.80[f]
OS1	4.15 ± 0.14[b]	2.93 ± 0.01[b]	13.51 ± 0.18[b]	6.59 ± 0.48[b]	6.00 ± 7.05[b]
OS2	4.50 ± 0.10[c]	2.93 ± 0.02[b]	12.59 ± 0.27[b]	3.89 ± 0.34[c]	7.40 ± 4.80[c]

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$)
RPMF – Raw pearl millet flour, FPMF – Fermented pearl millet flour, MPMF – Malted pearl millet flour, MLP – Moringa leaf powder, OS1 – 15% MLP, 30% MPMF, 55% FPMF, OS2 – 15% MLP, 55% MPMF, 30% FPM.

The moisture content of fermented, malted pearl millet and moringa leaf composite instant flours ranged from 4.15 to 4.50%, values that are substantially low when compared to the 9.47% of RPMF. The moisture content range for the inputs was 2.89 to 7.87%, with FPMF exhibiting a very low value, possibly aided by the freeze-drying process applied to render the fermented slurry into flour. The low moisture content acquired on the composite instant flours, OS1 and OS2, will favour their keeping quality.

The ash content of both fermented, malted pearl millet and moringa leaf composite instant flours was 2.93 g translating into a 75% increase from the 1.67 g/100 g of RPMF. El-Fatah *et al.* (2013:1075) and Nour & Ibrahim (2016:673) reported even higher ash content increases of 92% and 240% upon compositing MLP with cereal flours at ratios of 7.5% and 15%, respectively. The increase can be attributed to compositing fermented and malted pearl millet with MLP whose ash content was 10.76 g/ 100 g. The ash content of MLP was higher than the 7.64 g/100 g reported by Moyo *et al.* (2011:12927) but comparable to the 10.9 and 11.50 g/100 g stated by Teixeira *et al.* (2014:52) and Isitua *et al.* (2015:11), respectively. The overall impact of MLP on the ash content of pearl millet could help alleviate mineral deficiencies in consumers.

The protein content of the fermented, malted pearl millet and moringa leaf composite instant flours ranged from 12.59 to 13.51 g/100 g, translating to a maximum increase of 22% from the 11.12 g/100 g for RPMF. The two protein contents for OS1 (13.51 g/100 g) and OS2 (12.59 g/100 g) were higher than the projected values of 12.41 g/100 g and 11.85 g/100 g, from the optimisation process,

respectively. The significant ($p \leq 0.05$) protein increase can be attributed to MLP with the highest protein content of 26.32 g/100 g when compared to other inputs, MPMF and FPMF with 9.98 g/100 g and 10.42 g/100 g, respectively. The protein content of MLP, 26.32 g/100 g, was very similar to the 26.28 g/100 g and 26.71 g/100 g values reported by Olusanya (2018:81) and Nour & Ibrahim (2016:673) respectively. Moyo *et al.* (2011:12927) and El-Fatah *et al.* (2013:1069) however, reported higher figures reaching 30.00 g/ 100 g in earlier studies. The protein content of the Fermented, malted pearl millet and moringa leaf composite instant flours was above the average protein content for selected common South African market instant cereal meals ranging from 5.00 to 16.10 g/100 g. The elevated protein content of pearl millet confirmed the first research hypothesis that indicated that compositing pearl millet with MLP would result in a significant improvement in the protein content of pearl millet food products. This result could therefore help alleviate protein-energy malnutrition for communities that rely on pearl millet as a staple food.

The fat content of the fermented, malted pearl millet and moringa leaf composite instant flours ranged from 3.89 to 6.59 g/100 g, translating to a 25% increase and 26% decrease in the fat content of OS1 & OS2, respectively, compared to RPMF at 5.28 g/100 g. Increasing the proportion of fermented pearl millet led to an increase in the total fat content of Fermented, malted pearl millet and moringa leaf composite instant flours as observed with the higher (6.59 g/100 g) fat content obtained in OS1 with 55% FMPF. Malting led to a significant ($p \leq 0.05$) reduction (18%) in the fat content of pearl millet, a result in line with the 12% and 26% reductions observed by Adebiyi *et al.* (2017:213) and Obilana (2013:70), respectively. The fat content of RPMF (5.28 g/100 g) was higher than the 3.98 g/100 g acquired by Obilana (2013:128) but is closely equivalent to the 5.53 g/100 g average of three cultivars reported by Kulthe *et al.* (2016:4673). MLP's fat content (2.71 g/100 g) was quite lower than the 4.50 g/100 g and 5.75 g/100 g reported by Offor *et al.* (2014:60) and Penalver *et al.* (2022:5) possibly due to varietal differences.

Compositing malted and fermented pearl millet with MLP led to a significant ($p \leq 0.05$) increase in the total sugar content of the Fermented, malted pearl millet and moringa leaf composite instant flours with a range of. 6.00 to 7.40 g/100 g when compared to RPMF's 1.10 g/ 100g. The MPMF, FPMF and MLP's total sugar content ranged from 2.00 to 4.70 g/100 g with FPMF recording the lowest whilst MLP had the highest figure although it was less than the 13.02 g/ 100 g reported by Thierry *et al.* (2013:853). MPMF and MLP contributed to elevated total sugar as found in OS2 with maximum contents of the two components. The elevated quantity of sugars in malted pearl millet could be attributed to starch hydrolysis by endogenous enzymes such as

amylase to produce free sugars, such as sucrose and maltose. The sugar profile and content of MPMF were similar to the findings of Badau *et al.* (2005:332) in their study on various pearl millet cultivars at 48 h of germination. Fermented pearl millet showed a similarly elevated total sugar content, although it was less than that of malted pearl millet, as starch was hydrolysed into simple sugars and subsequently organic acids by the action of lactic acid during fermentation.

The vitamin C content of fermented, malted pearl millet and moringa leaf composite instant flours ranged between 33.0 to 49.3 mg/100 g, values notably higher than the trace amounts in pearl millet (not exceeding 1 m g/100 g) reported by Nambiar *et al.* (2011:63) Pei *et al.* (2022:4). Ajantha *et al.* (2018:2480) reported 17.31 mg/100 g of vitamin C in MLP.

5.3.1.1 Amino acid profile of fermented, malted pearl millet and moringa leaf composite instant flours

Table 5.2 shows the amino acid content of fermented, malted pearl millet and moringa leaf composite instant flours alongside RPMF, MPMF, FPMF and MLP. Of the nine essential amino acids, eight were found with tryptophan being the exception.

Table 5.2 Amino acid content of fermented, malted pearl millet and moringa leaf composite instant flours (g/100 g)[1]

Amino acids	**RPMF**	**FPMF**	**MPMF**	**MLP**	**OS1**	**OS2**
Arginine	0.55 ± 0.07^{a}	0.55 ± 0.07^{a}	0.50 ± 0.00^{a}	1.60 ± 0.71^{b}	0.85 ± 0.35^{a,b}	0.70 ± 0.14^{a}
Histidine	0.25 ± 0.07^{a}	0.30 ± 0.00^{a}	0.30 ± 0.00^{a}	0.50 ± 0.14^{b}	0.35 ± 0.07^{a,b}	0.35 ± 0.07^{a,b}
Isoleucine	0.40 ± 0.00^{a}	0.50 ± 00^{a}	0.40 ± 0.00^{a}	1.10 ± 0.00^{b}	0.70 ± 0.28^{a}	0.55 ± 0.07^{a}
Leucine	0.95 ± 0.07^{a}	1.05 ± 0.70^{a}	0.85 ± 0.07^{a}	2.30 ± 0.00^{c}	1.60 ± 0.42^{b}	1.15 ± 0.07^{a}
Lysine	0.45 ± 0.35^{a,b}	0.30 ± 0.14^{a}	0.35 ± 0.21^{a}	1.00 ± 0.28^{b}	0.55 ± 0.07^{a,b}	0.45 ± 0.35^{a,b}
Methionine	0.40 ± 0.14^{a}	0.30 ± 0.14^{a}	0.25 ± 0.07^{a}	0.30 ± 0.14^{a}	0.60 ± 0.28^{a}	0.60 ± 0.28^{a}
Phenylalanine	0.90 ± 0.28^{a}	1.05 ± 0.49^{a,b}	0.90 ± 0.28^{a}	2.60 ± 0.57^{c}	1.85 ± 0.07^{b,c}	1.10 ± 0.14^{a,b}
Threonine	0.45 ± 0.07^{a}	0.55 ± 0.07^{a}	0.50 ± 0.00^{a}	1.50± 0.40^{b}	0.90 ± 0.42^{a,b}	0.70 ± 0.14^{a}
Tyrosine	0.45 ± 0.35^{a}	0.45 ± 0.07^{a}	0.40 ± 0.00^{a}	1.75 ± 0.07^{b}	0.70 ± 0.28^{a}	0.65 ± 0.21^{a}
Valine	0.50 ± 0.00^{a}	0.55 ± 0.07^{a}	0.45 ± 0.07^{a}	1.35 ± 0.07^{c}	0.85 ± 0.21^{b}	0.60 ± 0.00^{a}
Alanine	0.80 ± 0.00^{a}	0.90 ± 0.00^{a,b}	0.75± 0.07^{a}	1.85 ± 0.07^{c}	1.20 ± 0.28^{b}	0.90 ± 0.00^{a,b}
Asparagine	0.75 ± 0.07^{a}	0.80 ± 0.00^{a}	0.95 ± 0.07^{a}	2.65 ± 0.35^{b}	1.30 ± 0.42^{a}	1.00 ± 0.00^{a}
Serine	0.60 ± 0.00^{a}	0.55 ± 0.21^{a}	0.60 ± 0.00^{a}	1.55 ± 0.07^{b}	0.95 ± 0.35^{a}	0.70 ± 0.14^{a}
Glutamate	1.75 ± 0.21^{a}	1.85 ± 0.07^{a}	1.70 ± 0.14^{a}	3.35 ± 0.50^{b}	2.45 ± 0.78^{a,b}	1.85 ± 0.07^{a}
Proline	0.50 ± 0.14^{a}	0.50 ± 0.14^{a}	0.45 ± 0.07^{a}	1.00 ± 0.14^{a}	0.80 ± 0.42^{a}	0.55 ± 0.21^{a}

[1]Values are mean ± standard deviation. Means with different superscripts in each row differ significantly ($p \leq 0.05$) RPMF – Raw pearl millet flour, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, MLP – Moringa leaf powder, OS1 – 15% MLP, 30% MPMF, 55% FPMF, OS2 – 15% MLP, 55% MPMF, 30% FPM.

The lysine content of fermented, malted pearl millet and moringa leaf composite instant flours ranged from 0.45 to 0.55 g/100 g with RPMF at 0.45 g/100 g. Overall, there was no significant ($p \leq 0.05$) increase in lysine content with the addition of MLP at a maximum of 15%, although OS1 showed a 22% increase, with the resultant value of 0.55 g/100 g covering 10% of the WHO (2007) standard daily lysine requirements. Processing of pearl millet (malting and fermentation) seemingly reduced the lysine content by up to 33%, a trend that may have impacted the overall augmentation significance of MLP which had a significantly ($p \leq 0.05$) higher value of 1.00 g/100 g. The reduction of lysine during fermentation was unexpected, although it was not different from one of the findings presented by Osman (2011b:3). The lysine content of MLP was slightly lower than 1.54 g/100 g and 1.64 g/100 g which were reported by Juhaimi *et al.* (2017:5) and Moyo *et al.* (2011:12928), respectively. Moreover, these values were far less than the 5.71 g/100 g acquired by El-Fatah *et al.* (2013:1071). Lysine is key for the growth and maintenance of the body by contributing to protein synthesis, promoting adequate calcium absorption, and strengthening the immune system by supporting the production of antibodies (Moyo *et al*., 2011:12928; Kayitesi *et al*., 2012:840).

There was a 50% increase in the sulphur-containing methionine from 0.40 g/100 g (RPMF) to 0.60 g/100 g for both fermented, malted pearl millet and moringa leaf composite instant flours with resultant values covering about 27% of the WHO (2007) standard daily requirements. Contrary to expectations, RPMF recorded a higher methionine content (0.40 g/100 g) than MLP (0.30 g/100 g), although this was much lower than the 0.83 g/100 g reported by Anitha *et al.* (2020:79). The methionine content of MLP at 0.30 g/100 g was equal to the value reported by Moyo *et al.* (2011:12928) and slightly lower than the 0.56 g/100 acquired by Juhaimi *et al.* (2017:5). Methionine is involved in the synthesis of protein, its structure and functionality as well as the generation of intracellular antioxidants (J. Brosnan & Brosnan, 2006:1636; Nambiar *et al*., 2011:64; Colovic *et al*., 2018:3). Isoleucine and threonine increased by up to 75% and 100%, respectively, in the pearl millet and MLP composites. Other essential amino acids comprising leucine, phenylalanine, and valine also significantly ($p \leq 0.05$) increased particularly in OS1, probably due to the maximum MLP content.

5.3.1.2 Sugar content and starch digestibility of fermented, malted pearl millet and moringa leaf composite instant flours

Table 5.3 shows the sugar composition, and starch digestibility of fermented, malted pearl millet and moringa leaf composite instant flours, alongside inputs.

Table 5.3 Sugar composition and starch digestibility of fermented, malted pearl millet and moringa leaf instant flours (g/100 g)[1]

Sugars	**RPMF**	**FPMF**	**MPMF**	**MLP**	**OS1**	**OS2**
Fructose	0.20 ± 0.01^{a}	0.30 ± 0.01^{c}	0.30 ± 0.02^{c}	1.20 ± 0.01^{d}	1.00 ± 0.07^{b}	1.00 ± 0.06^{b}
Glucose	0.10 ± 0.00^{a}	1.70 ± 0.05^{c}	0.30 ± 0.08^{a}	0.30 ± 0.25^{a}	3.30 ± 0.14^{b}	3.30 ± 0.31^{b}
Sucrose	0.80 ± 0.00^{a}	0.00 ± 0.00^{b}	2.70 ± 0.00^{c}	2.00 ± 0.13^{d}	0.10 ± 0.03^{b}	0.10 ± 0.00^{b}
Maltose	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.20 ± 0.00^{a}	0.00 ± 0.00^{a}	1.30 ± 0.10^{b}	2.60 ± 0.26^{c}
Lactose	0.00 ± .00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.10 ± 0.01^{b}	0.10 ± 0.02^{b}
Galactose	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.20 ± 0.04^{c}	0.30 ± 0.01^{b}	0.30 ± 0.02^{b}
Trehalose	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
IVSD*	50.88 ± 1.13^{a,c}	53.85 ± 4.10^{c}	47.00 ± 2.58^{a,b}	45.80 ± 2.47^{b}	43.74 ± 2.78^{b}	44.56 ± 2.00^{b}

[1]Values are mean ± standard deviation. Means with different superscripts in each row differ significantly ($p \leq 0.05$). RPMF – Raw pearl millet flour, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, MLP – Moringa leaf powder, OS1– 15% MLP, 30% MPMF, 55% FPMF, OS2 – 15% MLP, 55% MPMF, 30% FPM, *IVSD – *in vitro* starch digestibility (Units expressed as mg maltose / g starch).

The fructose, glucose, and sucrose quantities for both OS1 and OS2 were all at par with values of 1.00, 3.30 and 0.10 g/100 g, respectively. Generally, there was a high proportion of simple sugars, particularly fructose and glucose in proportions of 72% and 54% for OS1 and OS2, respectively. The high proportion of simple sugars in the composite flours may be attributed to the effect of the increase in microbial amylase activity during the fermentation of pearl millet leading to the hydrolysis of starch into maltodextrins, and simple sugars which are subsequently converted to organic acids (Osman, 2011b:3). This phenomenon is consistent with the observation that OS1, with a higher proportion of fermented pearl millet (55%), equally had a higher proportion (72%) of simple sugars. MLP, with a fructose content of 1.20 g/100 g also contributed to the high proportion of simple sugars in the composite products. Maltose dominated the disaccharides content in pearl millet and MLP composite with OS1 and OS2 registering 1.30 g/100 g and 2.60 g/100, respectively, while sucrose and lactose were both found in trace quantities across both variants. The high prevalence of maltose can be attributed to wet cooking leading to the swelling of starch granules and rendering them highly susceptible to enzymatic hydrolysis (Reed *et al*., 2013:1079). Galactose content was 0.30 g/100 g for OS1 and OS2 owing to the 1.30 g/100 g found only in MLP across the inputs.

The *in vitro* starch digestibility values of OS1 and OS2 were quite similar at 43.75 mg and 44.56 mg maltose per 100 g starch. These values were lower than those obtained from the pearl millet and MLP inputs whose range was 45.80 to 53.85 g maltose per 100 g of starch with MLP and FPMF recording the lowest and highest values, respectively. The reduced *in vitro* starch digestibility could be due to starch retrogradation during cooling post the wet cooking process. The *in vitro* starch digestibility of malted pearl millet was slightly higher than the 41.93 mg maltose/100 g starch obtained by Obilana (2013:142). Fermentation and malting marginally increased and decreased the *in vitro* starch digestibility of pearl millet as similarly reported by Alka *et al.* (2012:69) and Obilana (2013:142), respectively. The reduction of *in vitro* starch digestibility in fermented, malted pearl millet and moringa leaf composite instant flours may not be adverse considering that this trend translates into a reduced glycemic index beneficial in managing maturity-onset diabetes and obesity (Nambiar *et al*., 2011:64; Shumoy & Raes, 2017:381; Patel *et al*., 2017:154).

5.3.1.3 Fatty acids composition of fermented, malted pearl millet and moringa leaf composite instant flours

Table 5.4 shows the fatty acid content of fermented, malted pearl millet and moringa leaf composite instant flours, alongside RPMF, MPMF, FPMF and MLP.

Table 5.4 Fatty acid composition of fermented, malted pearl millet and moringa leaf composite instant flours (g/100 g)[1]

	RPMF	FPMF	MPMF	MLP	OS1	OS2
Saturated Fat	1.76 ± 0.14^{a}	1.67 ± 0.07^{a}	1.42 ± 0.04^{a}	2.30 ± 0.29^{b}	2.43 ± 0.60^{b}	1.54 ± 0.13^{a}
Palmitic acid (C16:0)	1.33 ± 0.11^{a}	1.27 ± 0.05^{a}	1.06 ± 0.02^{b}	1.04 ± 0.11^{b}	1.40 ± 0.00^{a}	1.13 ± 0.09^{b}
Stearic acid (C18)	0.27 ± 0.03^{a}	0.25 ± 0.01^{a,c}	0.23 ± 0.01^{c}	0.18 ± 0.02^{d}	0.45 ± 0.01^{b}	0.23 ± 0.02^{c}
Arachidic (C20)	0.16 ± 0.01^{a}	0.14 ± 0.01^{a}	0.12 ± 0.01^{a}	1.07 ± 0.16^{b}	0.27 ± 0.09^{a}	0.19 ± 0.01^{a}
Monounsaturated Fat	1.41 ± 0.11^{a,b}	1.31 ± 0.06^{a,c}	1.13 ± 0.03^{a,c}	0.15 ± 0.03^{d}	1.69 ± 0.40^{b}	1.03 ± 0.11^{c}
Oleic acid (C18:1n9c)	1.41 ± 0.11^{a}	1.31 ± 0.62^{a,b,c}	1.13 ± 0.03^{a,c}	0.12 ± 0.01^{d}	1.62 ± 0.39^{b}	1.01 ± 0.07^{c}
Palmitoleic acid (C16:1)	N.D	N.D	N.D	0.03 ± 0.04^{a,b}	0.07 ± 0.02^{b}	0.03 ± 0.04^{a,b}
Polyunsaturated Fat	2.11 ± 0.16^{a}	1.90 ± 0.09^{d}	1.77 ± 0.03^{d}	0.26 ± 0.0^{e}	2.77 ± 0.00^{b}	1.32 ± 0.09^{c}
Linoleic (C18:2n9c)	2.11 ± 0.16^{a}	1.90 ± 0.09^{d}	1.77 ± 0.03^{d}	0.26± 0.03^{e}	2.77 ± 0.00^{b}	1.32 ± 0.09^{c}

[1]Values are mean ± standard deviation. Means with different superscripts in each row differ significantly ($p \leq 0.05$) RPMF – Raw pearl millet flour, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, MLP – Moringa leaf powder, OS1 – 15% MLP, 30% MPMF, 55% FPMF, OS2 – 15% MLP, 55% MPMF, 30% FPM, ND – Not detected.

OS1 had a higher proportion of saturated fat at 2.43 g/100 g when compared to the 1.54 g/100 g observed in OS2 owing to the optimisation process where the constraint of minimising saturated fat was employed. The 1.54 g/100 g acquired in OS2 was very comparable to the 1.25 g/100 g projected during the numerical optimisation process. Palmitic, stearic and arachidic acids accounted for up to 73, 19 and 12% respectively, of the total saturated fat, with the bulk of the arachidic acid contribution coming from MLP. The abundance of palmitic acid in pearl millet, contributing up to 25% of the total fatty acid content, was consistent with the findings from studies by Jukanti *et al*. (2016:320) and Tomar *et al*. (2021:7). MLP's lipid profile was dominated by arachidic acid accounting for 39% and palmitic acid at 38%, with stearic acid completing the 85% saturated fatty acid proportion of the total fatty acid content. The palmitic acid proportion of MLP was higher than the 25% and 11% reported by Amaglo *et al*. (2010:1052) and Moyo *et al*. (2011:12930), respectively. Overall, the mixture design methodology was successful in determining the ideal composition ratio of pearl millet and MLP components for achieving minimal saturated fat content as observed in OS2. Saturated fats have been for long associated with a high risk of coronary heart diseases by increasing low-density lipoprotein cholesterol although recent studies have indicated otherwise yet still recommending their moderate intake and replacement with unsaturated fats (Siri-Tarino *et al*., 2010:502; Hooper *et al*., 2015:2; Nettleton *et al*., 2017:31; Dinicolantonio & Keefe, 2018:4).

Monounsaturated fats for OS1 and OS2 ranged from 1.03–1.69 g/100 g, respectively. The oleic (omega 9) fatty acid was by far the abundant unsaturated fatty acid contributing up to 98% of the total saturated fat, with a complimentary trace portion of palmitoleic acid. The dominance of oleic acid in the fermented, malted pearl millet and moringa leaf composite instant flours was due to its abundance in pearl millet contributing up to 27% of the total fat fatty acid content. This finding was in line with Issoufou *et al*. (2013:503) and Tomar *et al*. (2021:7) with the latter reporting an average of 28% oleic acid across the total fat content of 87 pearl millet genotypes. The polyunsaturated fatty acid content, characterised by only linoleic acid, of the fermented, malted pearl millet and moringa leaf composite instant flours ranged between 1.32 and 2.77 g/100 g. Moreover, linoleic acid was the most abundant fatty acid contributing up to 42% of the total fat content, a proportion comparable to the 40% peak reported by Tomar *et al*. (2021:7). The linoleic acid proportion of MLP, accounting for 10% of total fatty acid content was closely comparable to the 11% reported by both Amaglo *et al*. (2010:1052) and Moyo *et al*. (2011:12930).

5.3.1.4 Mineral composition of fermented, malted pearl millet and moringa leaf composite instant flours

Table 5.5 shows the mineral composition of fermented, malted pearl millet and moringa leaf composite instant flours, RPMF, MPMF, FPMF and MLP. The calcium (Ca) content of the fermented, malted pearl millet and moringa leaf composite instant flours ranged between 381.5 to 414.8 mg/100 g translating into over 1200% increase from the 30.8 mg/100 g in RPMF. This may be attributed to the high Ca content (1477.0 mg/100 g) of MLP. The Ca content of the pearl millet and MLP composites cover about 41% of FAO & WHO (2004) recommended daily intake for adults. The Ca content of pearl millet (30.8 mg/100 g) was less but comparable to the 35.1 mg/ 100 g obtained by Obilana (2013:128) and much less than the 40.1 mg/ 100 g average reported by Iswarya & Narayanan (2016:394). Calcium is key for bone and teeth formation, providing rigidity to the skeleton with its ions playing many roles in metabolic processes, nerve, and muscle functioning, and clotting of blood (FAO & WHO, 2004; Akram *et al*., 2020:152).

The iron (Fe) content of the fermented, malted pearl millet and moringa leaf composite instant flours averaged 7.22 mg/100 g translating into over 100% increase owing to the high Fe content (37.52 mg/100 g) of MLP. The Fe content of the pearl millet and MLP composite flours covers a significant portion of FAO & WHO (2004) recommended daily intake of 9.1 to 19.6 mg at 15% bioavailability. RPMF's Fe content (3.51 mg/100 g) was lower than the 4.6 mg/100 g and 7.10 mg/100 g stated by Iswarya & Narayanan (2016:394) and Obilana (2013:128) respectively. The Fe content of MLP (37.52 mg/100 g) was higher than the 25.14 mg/100 g reported by Penalver *et al.* (2022:6). Iron plays a vital role in oxygen transportation to the tissues from the lungs by red blood cell haemoglobin, intercellular electron transportation and as an integrated part of enzyme systems in various tissues (FAO & WHO, 2004).

The magnesium (Mg) content of the fermented, malted pearl millet and moringa leaf composite instant flours ranged between 148.7 and 152.8 mg/100 g translating to over a 50% increase and covering about 60% of the FAO & WHO (2004) daily recommended intake. The Mg content increase may be attributed to the high Mg content in MLP which was higher than the 233.5 mg/100 g stated by Nour & Ibrahim (2016:674) but lower than the 301.1 mg/100 g reported by Penalver *et al.* (2022:6). On the other hand, the Mg content of RPMF was much lower than the 137.0 mg/100 g stated by Nambiar *et al.* (2011:63). Mg, as a co-factor of various enzymes, plays numerous roles in energy metabolic reactions, protein, RNA and DNA synthesis and upkeep of the electrical potential in nervous tissue FAO & WHO (2004).

Table 5.5 Mineral content of fermented, malted pearl millet and moringa leaf composite instant flours (mg/100 g)[1]

Minerals	RPMF	FPMF	MPMF	MLP	OS1	OS2
Calcium	30.8 ± 0.78[a]	26.7 ± 1.40[a]	31.0 ± 0.41a	1477.0 ± 46.97[c]	414.8 ± 13.4[b]	381.5 ± 14.4[b]
Copper	0.48 ± 0.01[a]	0.52 ± 0.00[b]	0.56 ± 0.08b	0.87 ± 0.08[c]	0.40 ± 0.01[a]	0.40 ± 0.01[a]
Iron	3.51 ± 0.04[a]	2.58± 0.06[a]	3.28 ± 0.53a	37.52 ± 6.10[b]	7.23 ± 0.46[a]	7.20 ± 0.36[a]
Magnesium	98.5 ± 0.86[a]	79.2 ± 2.91[d]	89.4 ± 1.84c	282.0 ± 9.29[e]	152.8 ± 1.81[b]	148.7 ± 1.60[b]
Manganese	1.21 ± 0.35[a]	0.95 ± 0.04[c]	1.09 ± 0.09c	3.19 ± 0.25[d]	1.71 ± 0.04[,b]	1.31 ± 0.01[b]
Potassium	435.9 ± 8.37[a]	368.3 ± 24.00[c]	343.6 ± 4.26c	897.7 ± 29.61[d]	479.8 ± 1.23[b]	450.1 ± 5.08[a]
Sodium	42.8 ± 1.54[a,e]	39.4 ± 4.78[a]	115.9 ± 9.14d	55.11 ± 13.48[e]	61.4 ± 1.64[b]	74.9 ± 0.57[c]
Zinc	2.14 ± 0.16[a]	1.83 ± 0.12[a]	2.15 ± 0.39a	3.27 ± 0.95[b]	1.86 ± 0.06[a]	1.94 ± 0.14[a]

[1]Values are mean ± standard deviation. Means with different superscripts in each row differ significantly ($p \leq 0.05$) RPMF – Raw pearl millet flour, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, MLP – Moringa leaf powder, OS1 – 15% MLP, 30% MPMF, 55% FPMF, OS2 – 15% MLP, 55% MPMF, 30% FPM

Other minerals comprising manganese (Mn), potassium (K) and sodium (Na) increased by up to 41, 10, and 75%, respectively, with increases in Mn and K, attributed to their higher proportions in MLP whilst Na boost came from malted pearl millet. The Na spike was most likely due to the residual sodium hydroxide solution employed during the steeping phase of the malting process. Overall, compositing pearl millet with MLP resulted in significant ($p \leq 0.05$) increases in minerals in pearl millet, confirming the second hypothesis that indicated compositing pearl millet with MLP significantly improves the mineral content and nutrient density of pearl millet. This outcome affirms the high potential of MLP in alleviating mineral deficiencies for pearl millet consumers. The bioavailability of the minerals in the composite food matrix is another factor to consider although the substantial overall increases observed may as well compensate for any shortcomings thereof.

5.3.2 Physicochemical properties of of fermented, malted pearl millet and moringa leaf composite instant flours

5.3.2.1 Pasting properties

The pasting properties of fermented, malted pearl millet and moringa leaf instant flours are summarised in Table 5.6.

Table 5.6 Pasting properties of of fermented, malted pearl millet and moringa leaf composite instant flours[1]

Sample ID	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)	Pasting Temp. °C	Peak Time (min)
RPMF	281.67 ± 0.58^{a}	260.00 ± 2.00^{a}	21.67 ± 2.08^{a}	866.67± 17.62^{a}	606.67 ± 15.63^{a}	89.93 ± 0.03	5.00 ± 0.00^{a}
FPMF	830.00 ± 83.26^{c}	803.00 ± 75.90^{c}	27.00 ± 7.81^{a}	1435.33 ± 136.08^{c}	632.33 ± 60.58^{a}	87.85 ± 0.57	6.95 ± 0.04^{d}
MPMF	34.67 ± 2.89^{b}	23.67 ± 1.15^{b}	11.00 ± 1.73^{b,c}	48.00 ± 2.65^{b}	24.33 ± 1.53^{b}	ND	3.8 ± 0.00^{c}
MLP	27.00 ± 3.61^{b}	16.33 ± 3.51^{b}	10.66 ± 0.58^{b,c}	30.00 ± 3.61^{b}	13.67 ± 0.58^{b}	ND	3.93 ± 0.17^{c}
OS1	45.67± 2.08^{b}	30.33 ± 0.58^{b}	15.33 ± 1.53^{b}	63.67 ± 1.15^{b}	33.33 ± 0.58^{b}	ND	1.09 ± 0.03^{b}
OS2	27.67 ± 1.52^{b}	23.00 ± 0.00^{b}	4.67 ± 1.53^{c}	46.33 ± 1.15^{b}	23.33 ± 1.15^{b}	ND	1.22 ± 0.10^{b}

[1]Values are mean ± standard deviation. Means with a different superscript in each column differ significantly ($p \leq 0.05$), ND – Not Determined, RPMF – Raw pearl millet flour, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, MLP – Moringa leaf powder, OS1 – 15% MPL, 30% MPMF, 55% FPMF, OS2 – 15% MPLF, 55% MPMF, 30% FPMF.

The peak viscosity of the fermented, malted pearl millet and moringa leaf composite instant flours OS1 and OS2 ranged from 27.67 to 45.67 Cp, the final viscosity ranged between 46.33 and 63.67 Cp whereas the peak viscosity ranged between 46.33 to 48.00 Cp. Trough viscosity, breakdown viscosity and setback viscosity ranged from 23.00 to 30.33 Cp, 4.67 to 15.33 Cp and 23.33 to 33.33 Cp, respectively. The pasting temperatures were not determined for the two formulations in the RVA. This could be related to the malting and the initial heat treatment (cooking) disintegrating the carbohydrate components and therefore rendering the flours ready for reconstitution with lower temperature liquids for making a beverage or porridge. Peak time ranged from 1.09 to 1.22 Cp.

The inputs' (FPMF, MPMF and MLP) peak viscosity ranged from 27.00 to 281.67 Cp, breakdown viscosity from 10.66 to 27.00 Cp and final viscosity from 30.00 to 866.67 Cp. The pasting temperature ranged from 87.85 to 89.93°C for FMPF and RPMF, whilst it was not determined on MLP and MPMF, the latter being reported to be at a lower value of 30°C. Fermentation increased the viscosity of pearl millet significantly ($p \leq 0.05$) as also reported by Akinola *et al.* (2017:5). The RPMF viscosity results were similar to Gull *et al.* (2016:99). Malting, on the contrary, significantly decreased the viscosity of pearl millet, in line with the findings of Obilana (2013: 106) who attributed the trend to the degradation of starch by amylases.

The low viscosity of the fermented, malted pearl millet and moringa leaf composite instant flours, imparted by MPMF and MLP, indicates their reduced ability to form a viscous paste associated with higher nutrient density (Filli *et al*., 2012:3; Awolu *et al*., 2018:253). This was evident during the cooking of the raw composite pearl millet and MLP flours for numerical optimisation whereby recipes with higher proportions of malted flour required 33% less water to achieve desired simmering consistency when compared to recipes with more fermented flour. Pelembe *et al.* (2002a:125) further postulated that the decrease in viscosity could be advantageous for infants or anyone who requires spoonable viscosity, with retention of high nutrient or energy density. As such, only malting improved the pasting properties of the pearl millet and MLP composite flours, that is, partially confirming the third research hypothesis that indicated the application of malting and fermentation to improve the pasting properties of the pearl millet and MLP composite.

5.3.2.2 Moisture content and water interaction properties

Table 5.7 shows the moisture content of fermented, malted pearl millet and moringa leaf composite instant flours, alongside RPMF, MPMF, FPMF and MLP.

Table 5.7 Moisture content of fermented, malted pearl millet and moringa leaf composite instant flours[1]

Sample ID	Moisture Content (%)
RPMF	9.47 ± 0.04[a]
FPMF	2.89 ± 0.04[e]
MPMF	7.87 ± 0.06[d]
MLP	7.11 ± 0.12[f]
OS1	4.15 ± 0.14[b]
OS2	4.50 ± 0.10[c]

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$) RPMF – Raw pearl millet flour, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, MLP – Moringa leaf powder, OS1 – 15% MLP, 30% MPMF, 55% FPMF, OS2 – 15% MLP, 55% MPMF, 30% FPMF.

Moisture content ranged from 4.15 to 4.50% for the fermented, malted pearl millet and moringa leaf composite instant flours OS1 and OS2, values that are quite low when compared to the 9.47% of RPMF. The range for the inputs, was 2.89 to 7.87%, with FPMF exhibiting a very low moisture content, possibly aided by freeze-drying.

The water interaction properties of fermented, malted pearl millet and moringa leaf composite instant flours, alongside RPMF, MPMF, FPMF and MLP are summarised in Table 5.8.

Table 5.8 Water interaction properties of fermented, malted pearl millet and moringa leaf composite instant flours[1]

Sample ID	**WAI (%)**	**WHC (g/g)**	**WSI (%)**	**Aw (A_w)**
RPMF	249.40 ± 10.14[a]	1.29 ± 0.02[a]	7.71 ± 0.93[a]	0.5100 ± 0.01[a]
FPMF	248.94 ± 3.53[a]	1.19 ± 0.09[a]	13.44 ± 1.42[d]	0.1967 ± 0.06[d]
MPMF	272.78 ± 13.23[a]	1.27 ± 0.06[a]	13.10 ± 1.54[d]	0.4200. ± 0.01[c]
MLP	423.46 ± 12.96[c]	2.86 ± 0.09[d]	30.03 ± 2.76[c]	0.5133 ± 0.01[a]
OS1	481.94 ± 7.39[b]	3.40 ± 0.07[b]	21.81 ± 1.85[b]	0.3300 ± 0.00[b]
OS2	418.90 ± 27.56[c]	3.17 ± 0.06[c]	32.98 ± 3.58[c]	0.3267 ± 2.00[b]

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$) WAI – Water absorption index, WHC – Water holding capacity, WSI – Water solubility index, Aw – Water activity, RPMF – Raw pearl millet flour, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, MLP – Moringa leaf powder, OS1 – 15% MLP, 30% MPMF, 55% FPMF, OS2 – 15% MLP, 55% MPMF, 30% FPMF.

The water absorption index (WAI) ranged from 418.90 to 481.94% for the fermented, malted pearl millet and moringa leaf composite instant flours. For the RPMF and inputs of the composite flours, WAI ranged from 248.94 to 423.46%. The WAI values

of RPMF and MPMF were similar to the findings of Obilana (2013:98). WAI has been ascribed to amylose/amylopectin ratios in the flour with higher amylose directly correlated to higher WAI (Pelembe *et al*., 2002b:124). Malting did not have a significant ($p \leq 0.05$) impact on WAI, as per Obilana (2013:98), although it has been reported to increase WAI as the synthesis of structural components such as hemicellulose and cellulose occurs during germination (Obadina *et al*., 2017:4444). The higher WAI of pearl millet and MLP composite flours may then be attributed to MLP, which had a high WAI (423.46%) and reportedly has a high crude fibre content that peaking just above 10% (El-Fatah *et al*., 2013:1069; Nour & Ibrahim, 2016:673). Moreover, the gelatinisation of starch, as postulated by Obilana (2013:99), results in more open or enlarged starch granules that readily absorb water and paste and therefore rendering the cooked composite flours more suitable for cold to warm water reconstitution to prepare an instant beverage or porridge.

Water holding capacity (WHC) ranged from 3.17 to 3.40 g/g for the fermented, malted pearl millet and moringa leaf composite instant flours, values significantly higher than those of inputs (ranging from 1.19 to 2.86 g/g). The higher WHC of the composite food flours may be linked to MLP which exhibited a high value of 2.86 g/g, possibly due to the high crude fibre. Malting and fermentation did impact the WHC of pearl millet. Water solubility index (WSI), ranged from 21.81 to 32.98% for the fermented, malted pearl millet and moringa leaf composite instant flours, whilst the inputs' WSI ranged from 13.10 to 30.03% with RPMF at 7.71%. The WSI for RPMF (7.71%) and MPMF (13.10%) were comparable to those of Obilana (2013:98) and Obadina *et al.*, 2017 (2017:4448), with RPMF values of 5.14% and 5.13% and, MPM values of 12.16% and 12.62%, respectively. These authors also reported a significant increase in WSI due to malting pearl millet, a trend observed in the present study, with Obadina *et al.* (2017:4448) ascribing this phenomenon to depolymerisation of starch due to enzymatic action in crystalline regions of pearl millet leading to elevated hygroscopicity. Fermentation of pearl millet also resulted in a significant ($p \leq 0.05$) increase in WSI, a trend similar to those reported by Onweluzo & Nwabugwu (2009:741) who attributed this to hydrolysis of high molecular weight carbohydrates and proteins to simpler and more soluble during fermentation. WSI serves as an indication of starch degradation and dextrinisation and ultimately the amount of soluble and digestible materials in pearl millet. Therefore, a higher WSI would be desirable for flours used to prepare instant meals by reconstituting with water.

Water activity (A_w) for the fermented, malted pearl millet and moringa leaf composite instant flours ranged between 0.3267 and 0.3300, a value that is significantly lower than that of RPMF. Moisture content and water activity are

generally related to keeping quality or shelf life, with flours ideally required to have low values for the two parameters. A combination of low moisture content and low water activity limits available water for microbial activity and consequently spoilage, imparting stability and reducing chances of rancidity, and ultimately leading to a longer shelf-life product (Akinola *et al*., 2017:3; Awolu *et al*., 2018:250).

5.3.2.3 Bulk Density

Table 5.9 shows bulk densities of the fermented, malted pearl millet and moringa leaf composite instant flours alongside RPMF, MPMF, FPMF and MLP.

Table 5.9 Bulk density of fermented, malted pearl millet and moringa leaf composite instant flours[1]

Sample ID	Bulk Density (kg/m^3)
RPMF	55.88 ± 0.67^a
FPMF	46.71 ± 0.39^c
MPMF	52.04 ± 0.47^a
OS1	32.31 ± 0.67^b
OS2	33.21 ± 0.25^b

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$), RPMF – Raw pearl millet flour, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, OS1 – 15% MLP, 30% MPMF, 55% FPMF, OS2 – 15% MLP, 55% MPMF, 30% FPMF.

The bulk density of fermented, malted pearl millet and moringa leaf composite instant flours ranged from 32.31–33.21 kg/m^3 indicating a reduction of up to 45% from RPMF's 58.88 kg/m^3. James *et al.* (2018:787), in the production of a pearl millet infant formula, indicated that a lower bulk density promotes the digestibility of pearl millet products. In the same study, the reduction of bulk density was attributed to fermentation and germination causing starch and peptide linkage hydrolysis, trends that were also observed in this study as per Table 5.9. On the contrary, the decrease in bulk density may not be desirable as packaging, storage and transportation requirements consequently increase.

5.3.3 Total phenolic content and antioxidant properties of fermented, malted pearl millet and moringa leaf composite instant flours

Table 5.10 illustrates the total phenolic content and antioxidant properties of fermented, malted pearl millet and moringa leaf composite instant flours alongside RPMF, MPMF, FPMF and MLP.

Table 5.10 Antioxidant properties of fermented, malted pearl millet and moringa leaf composite instant flours

Sample ID	TPC (mg GAE/100 g)	ORAC (µmol TE/100 g)	FRAP (µmol AAE/100 g)
RPMF	284.62 ± 9.27[a]	8127.88 ± 709.05[a]	1266.47 ± 49.80[a]
FPMF	342.57 ± 29.60[c]	8959.10 ± 46.56[a]	1161.87 ± 46.56[a]
MPMF	315.90 ± 4.07[a,c]	9334.97 ± 188.15[a]	1302.19 ± 188.15[a]
MLP	1482.56 ± 54.00[d]	19779.54 ± 264.86[c]	5820.32 ± 53.76[c]
OS1	477.44 ± 4.62[b]	11048.90 ± 553.28[b]	1587.92 ± 154.66[b]
OS2	513.33 ± 23.50[b]	11700.11 ± 1051.00[b]	1677.21 ± 26.88[b]

[1]Values are mean ± standard deviation. Means with different superscripts in each column differ significantly ($p \leq 0.05$). TPC – Total phenolic content (GAE – Gallic acid equivalent), ORAC – Oxygen radical absorbance capacity (TE – Trolox equivalent), FRAP – Ferric reducing antioxidant power (AAE – Ascorbic acid equivalent). RPMF – Raw pearl millet flour, MPMF – Malted pearl millet flour, FPMF – Fermented pearl millet flour, MLP – Moringa leaf powder, OS1 – 15% MLP, 30% MPMF, 55% FPMF, OS2 – 15% MLP, 55% MPMF, 30% FPMF.

Total phenolic content (TPC), oxygen radical absorbance capacity (ORAC), and ferric reducing antioxidant power (FRAP) respectively ranged from 477.44 to 513.33 mg GAE/100 g, 11048.90 to 11700.11 µmol TE/100 g and 1587.92 to 1677.21 µmol AAE/100 g for the composite flours OS1 and OS2. All antioxidant properties of fermented, malted pearl millet and moringa leaf composite instant flours were significantly ($p \leq 0.05$) superior to those of raw pearl millet. This can be attributed to the addition of MLP that presented significantly ($p \leq 0.05$) higher values on all antioxidant properties with TPC, ORAC and FRAP at 1482.56 mg GAE/100 g, 19779.54 µmol TE/100 g and 5820.32 µmol AAE/100 g, respectively, compared to raw, malted, and fermented pearl millet flours. The TPC value for MLP (1482.56 mg GAE/100 g) was, however, lower than the 3290 mg GAE/ 100 g reported by Penalver *et al.* (2022:7). Malted and fermented pearl millet flour TPC, ORAC and FRAP ranged from 315.90 to 342.57 mg GAE/100 g, 8959.10 to 9334.97 µmol TE/100 g and 1161.87 to 1302.19 µmol AAE/100 g, respectively.

Despite some phenolic compounds, particularly phytates from cereal grains, being known to present anti-nutritional properties, the greater portion of these compounds detected in the pearl millet and MLP composites were of MLP origin. MLP phenolic compounds have, on the hand, been reported to play key roles in the human body comprising anti-carcinogenic, immunomodulatory, anti-diabetic and antiatherogenic functions (Gebregiorgis, 2016:187; Razis *et al*., 2014:8571; Toma & Deyno, 2014:222). The high ORAC and FRAP values of fermented, malted pearl millet and moringa leaf composite instant flours are further indications of the elevated

antioxidant capacities presenting disease management and also imparting shelf-life preservation (Chandrasekara & Shahidi, 2011:435; Pushparaj & Urooj, 2014:60).

5.4 Conclusion

MLP was effective as a food fortificant with the significant increases observed in protein and ash content of the fermented, malted pearl millet and moringa leaf composite instant flours. Fermentation also led to a significant increase in protein content, a result not achieved through malting. Malting improved the pasting properties of pearl millet while fermentation on the contrary led to a huge increase in the viscosity of PM. The two contrasting effects did not significantly affect the pasting properties of the fermented, malted pearl millet and moringa leaf composite instant flours. Malting did not have a significant effect on the bulk density of pearl millet flour while fermentation lead to a decrease instead. Malting, fermentation, and augmenting pearl millet with MLP improved the water solubility index of the fermented, malted pearl millet and moringa leaf composite instant flours. Compositing fermented and malted pearl millet with MLP led to a significant increase in total phenolic content and a substantial improvement in the antioxidant properties of the composite instant flours.

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CHAPTER SIX
GENERAL SUMMARY AND CONCLUSION

This study sought to improve the nutritional, biochemical and physicochemical properties of pearl millet food products by employing malting, fermentation, and fortification. *Moringa oleifera* leaf powder (MLP) was used to fortify the malted and fermented pearl millet composite flour.

The objectives of the study were to:

1. Determine an optimum compositing ratio(s) of malted pearl millet flour, fermented pearl millet flour and MLP to attain a maximum protein content and or minimum saturated fat content.
2. Establish the impact of malting and fermentation on the nutritional, biochemical and physicochemical properties of pearl millet.
3. Produce fermented, malted pearl millet and moringa leaf composite instant flours based on mixture design and optimisation outcomes.
4. Assess the impact of compositing pearl millet with MLP on the nutritional, biochemical and physicochemical properties of the composite food products.

For the first objective, response surface methodology using mixture design and numerical optimisation was employed to find optimal proportions for each of the inputs (malted and fermented pearl millet flour and MLP) in the composite flours. Mixture design generated twelve mixtures with varying ratios of fermented pearl millet flour (FPMF), malted pearl millet flour (MPMF) ranging between 30–65% and MLP content ranging between 5–15% which were analysed for protein, fat and total phenolic content. The data obtained from the analysis was fitted to a linear model, which was suitable for explaining variation for total protein and saturated fat. Two final formulations were generated through the optimisation process, (1) 15:30:55 MLP, MPMF and FPMF, respectively, with 12.41% protein and 1.49% saturated fat for maximising protein with the desirability of 0.865 and (2) 15:55:30 MLP, MPMF and FPMF, respectively, having 11.84% protein and 1.25% saturated fat for maximising protein and minimising saturated fat with the desirability of 0.625. Overall, it was observed that increasing MLP increased the protein content as hypothesised.

Analysing the nutritional, biochemical and physicochemical properties/trends across different stages of fermentation and malting highlighting optimal periods for each parameter was carried out to achieve the 2nd objective of this research project. Fermenting pearl millet flour resulted in a significant ($p \leq 0.05$) increase in the protein content while the lysine content decreased slightly although not significant ($p \leq 0.05$). Malting however led to slight but not significant ($p \leq 0.05$) decreases in both the

protein and lysine content of pearl millet flour. Regarding physicochemical aspects, malting yielded desired results reducing the viscosity of pearl millet flour which translated into increased solids concentration in gruels and consequently higher nutrient density. On the other hand, fermentation increased the viscosity of pearl millet flour instead. Malting and fermentation improved the water solubility of pearl millet flour promoting reconstitutability of the instant composite flours. An increase in total phenolic content was observed in both malting and fermentation correlating with the improvement of the antioxidant properties of the respective pearl millet flours. Tannins were not detected across all the pearl millet flours.

The third objective was achieved by blending the fermented and malted pearl millet flours, as per the results of numerical optimisation. The blends were wet cooked and cooled down before the addition of MLP at <50°C and subsequent mixing for uniform distribution. The final pastes were cooled down, freeze-dried, and milled before packaging and storage at less than 5°C.

In achieving the fourth and final objective, the two final fermented, malted pearl millet and moringa leaf composite instant flours' nutritional content and profile were determined. It was observed that compositing fermented and malted pearl millet with MLP resulted in significant ($p \leq 0.05$) protein increases of up to 22%. The lysine content, although not significant ($p \leq 0.05$), also increased by up to 22%. Ash or mineral content increased by up to 75% with calcium, iron and magnesium content of pearl millet increased by over 1200%, and up to 106% and 37%, respectively. The vitamin C content of fermented, malted pearl millet and moringa leaf composite instant flours peaked at 49.3 mg/100 g, a result that is far better than the trace amounts (not exceeding 1 mg/100 g) reported by other researchers. In addition to the nutritional improvements observed, MLP also led to an overall increase in total phenolic content (TPC) of up to 45%. The TPC increase correlated with improvements in antioxidant properties indicating the impact of MLP in boosting the cereal's phytochemical profile.

The following conclusions were drawn from this study:

1. Fermentation yielded a significant ($p \leq 0.05$) increase in the protein content of pearl millet, a result that was not translated into the lysine content. Malting did not yield any significant changes to both the protein and lysine content.
2. Malting led to several improvements in the pasting properties of pearl millet, that is, most notably in the reduction of viscosity. The reduction of viscosity during malting was favourable as it translated into increased solids concentration and consequently higher nutrient density.

3. Both malting and fermentation led to an increase in total phenolic content and improvement in antioxidant properties in pearl millet flour, which was contrary to the research expectations.
4. Mixture design and numerical optimisation were effective in determining the optimum recipe for maximising protein and/ or minimising saturated fat with desirability peaking at 0.867.
5. Compositing pearl millet with MLP was found effective in augmenting the nutritional profile of pearl millet most notably protein and mineral content.
6. The addition of MLP to pearl mill*et al*so led to substantial improvement in the phytochemical content of pearl millet as observed in increases in TPC and antioxidant properties.
7. Literature information from this study contributed to the SAAFoST's FST Magazine April 2021, Volume 10, Issue 1.

The following challenges and limitations were experienced in the study:

1. Preserving the integrity of samples extracted from early malting stages until analysis was carried out, hence the missing 6 h and 12 h interval samples due to microbial spoilage.
2. It was not possible to conduct a sensory evaluation of the final products due to the Covid-19 pandemic and the delays led to the deterioration of all final products and inputs thereof while in storage for over 24 months. As such, consumer acceptability could not be investigated, although ethical clearance had been obtained.

The following areas related to the study could be of interest:

1. Determining the protein digestibility and consequently, protein digestibility-corrected amino acid score (PDCAAS) could be of use to further dissect the quality of protein contributed by MLP.
2. Determining the extractability of minerals that were observed to increase in content because of compositing pearl millet with MLP, to gather an indication of their availability for absorption in the human body.

APPENDIX A: Approved ethical clearance

Cape Peninsula
University of Technology

P.O. Box 1906 • Bellville 7535 South Africa •Tel: +27 21 953 8677 (Bellville), +27 21 460 4213 (Cape Town)

Office of the Chairperson Research Ethics Committee	Faculty of Applied Sciences

The Faculty Research Committee, in consultation with the Chair of the Faculty Ethics Committee, have determined that the research proposal of **F Sibanda (214332764)** for research activities related to a project undertaken for an **MTech: Food Technology** at the Cape Peninsula University of Technology, **does** require ethical clearance.

As such, this ethical clearance is issued on the basis that due diligence will be taken when involving human subjects. Ethical clearance given was on the basis that, all the required/requested information complied with minimum standards for ethical clearance.

Title of dissertation/ thesis:	Nutritional, biochemical and physicochemical properties of pearl millet / moringa oleifera composite food products

Comments (Add any further comments deemed necessary, eg permission required)
Research activities are restricted to those detailed in the research proposal.
Data/Sample collection/Participant consent permission required for this study.

Signed: Chairperson: Research Ethics Committee	Date 29/MAY/2017

Cape Peninsula
University of Technology

Statement of Permission

Data/Sample collection/Permission/Informed consent required for this study.

Surname & name	Faith Sibanda
Student Number	214332764
Degree	MTech: Food Technology
Title	Nutritional, biochemical and physicochemical properties of pearl millet / moringa oleifera composite food products
Primary Investigator	Dr. A. Obilana
FRC Signature	
Date	29/May/2017

APPENDIX B: Research outputs published and or presented at national and international conferences

1. Sibanda, F., Obilana A.O. & Jideani, V. A. (2018). Composite of fermented and malted pearl millet flour with Moringa oleifera leaf powder for optimum protein using mixture design, U6 Consortium 2nd International Conference, Cape Town, South Africa, 4 - 6 September 2018. (Oral presentation).

2. Sibanda, F., Obilana A.O. & Jideani, V. A. (2021). Nutritional, biochemical and physicochemical properties of pearl millet and *Moringa oleifera* composite food products. South African Food Science and Technology Sorghum FST Magazine, Volume 10(1), April 2021. (Published Magazine Article)

APPENDIX C: Manuscripts submitted for publication in peer-reviewed journals

Gmail faith sibanda <faithsibanda73@gmail.com>

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1 message

Food Science and Technology International <onbehalfof@manuscriptcentral.com> Sun, Jan 22, 2023 at 9:42 AM
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To: faithsibanda73@gmail.com, obilanaa@cput.ac.za, jideaniv@cput.ac.za

22-Jan-2023

Dear Mr. Sibanda:

Your manuscript entitled "Nutritional, biochemical and functional properties of pearl millet and Moringa oleifera leaf powder composite meal powders." has been successfully submitted online and is presently being given full consideration for publication in Food Science and Technology International.

Your manuscript ID is FSTI-23-0058.

You have listed the following individuals as authors of this manuscript:
Sibanda, Faith; Obilana, Anthony; Jideani, Victoria A.

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Shalini Lakhera
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fsti@sagepub.com

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Manuscript ID
FSTI-23-0058

Title
Nutritional, biochemical and functional properties of pearl millet and Moringa oleifera leaf powder composite meal powders.

Authors
Sibanda, Faith
Obilana, Anthony
Jideani, Victoria A.

Date Submitted
22-Jan-2023