Nutritional and functional properties of Bambara groundnut and *Moringa oleifera* leaf protein complex in a ready-to-use therapeutic food (RUTF)

by

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DECLARATION

I, Olawumi Oluwakemi Adewumi, hereby declare that the content of this dissertation/thesis is my original work and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

O O. ADEWUMI	13/11/2021
Signed	**Date**

ABSTRACT

This research aimed to produce Bambara groundnut-*Moringa oleifera* leaf protein complex (BAMOLP), evaluate its physical, nutritional, and functional properties to establish suitability as a functional ingredient in the production of ready-to-use therapeutic food. Protein isolates were extracted from Bambara groundnut flour (BGNF) and *Moringa oleifera* leaf powder (MOLP). The alkaline/isoelectric extraction method was used for the extraction of protein from BGNF, while heat treatment was used for protein extraction from MOLP. Phase behaviour of Bambara groundnut protein isolate (BGNPI) and *Moringa oleifera* protein isolate (MOLPI) was investigated to study their interaction and stability in a water system. BGNPI and MOLPI were varied at two levels each based on a 2^2 factorial experimental design augmented with centre points comprising of BGNPI (1, 5, 9% w/v) and MOLPI (5 to 4.5% w/v) as independent variables in 100% distilled water. Compositions obtained from 2^2 factorial experimental design were analysed for stability, particle size, and syneresis by using Turbiscan MA 2000, a zetasizer Malvern Instruments (NanoZS) and visual observation respectively. Eleven (11) compositions were established, and the effects were determined on the equilibrium backscattering (BS) flux as the stability indicator for the protein mixture. The BS profiles obtained from the Turbiscan stability analysis by using Turbiscan MA 2000 revealed Sedimentation, creaming, and flocculation were the destabilisation mechanism of the protein mixture. Experimental data obtained from stability index, syneresis (%), and particle size were used to determine the most stable composition of the protein mixtures. A protein mixture with 9% BGNPI and 2% MOLPI was established as the optimal mix for the production of BAMOLP. An investigation of physical, functional, and nutritional characteristics of Bambara groundnut protein isolates (BGNPI), *Moringa oleifera* leaf protein isolates (MOLPI), and their protein complex (BAMOLP) was carried out. The protein isolates and their complex were non-newtonian and pseudoplastic. The oil absorption capacity (OAC) of BGNPI, MOLPI, and BAMOLP was 2.26, 0.89, and 0.95 g/g respectively while the water absorption capacity (WAC) for BGNPI, MOLPI, and BAMOLP were 1.31, 1.5, and 1.22 g/g. The WAC and swelling capacity of the BGNPI, MOLPI, and BAMOLP increased with temperature from 60 to 90^{o}C. The foaming capacity was pH dependant. BAMOLP exhibited a higher emulsifying capacity and stability than BGNPI and MOLPI. The protein, fat, ash, carbohydrate, and moisture content of the protein isolates and complex ranged from 39.42 to 63.51%, 2.19 to 11.52%, 1.60 to 7.09%, 24.07 to 51.29%, and 2.61 to 9.57% respectively and significantly ($p < 0.05$) differed from one another. The results show that BAMOLP exhibits a better amino acid profile, rheological and chemical properties than its precursors: BGNPI and MOLPI. Furthermore, a ready-to-use therapeutic food (RUTF) using BAMOLP, oats, millets, egusi, MOLP, and other ingredients were simulated using the mixture preparation procedure in Superpro Designer. BAMOnut snack bars were assessed for physicochemical, nutritional, and sensory properties. Three

ready-to-use therapeutic bars produced were BAMOnut-OB3 (BAMOnut Bar enriched with oats and 3% BAMOLP, BAMOnut-MB2 (BAMOnut Bar enriched with millets and 2% BAMOLP), and BAMOnut-OMB5 (BAMOnut Bar enriched with oats, millets, and 5% BAMOLP). The BAMOnut snack bars were dark-yellowish, and less saturated since their chroma ranged from 11.70 to 20.83. Eleven mineral components (sodium, magnesium, phosphorous, potassium, calcium, iron, nickel, copper, zinc, selenium, iodine) were identified in the snack bars. There was no significant difference in the texture of the BAMOnut snack bars but the formulation with a higher concentration of MOLP has a lower sensory rating in appearance, colour, aroma, taste, and overall acceptability. Moisture (4.9%), protein (14.1%), fat (19.3%), CHO (59.7%), and energy (468.6 Kcal/100 g), of BAMOnut-OB3, compares favourably with the requirement for RUTF (2.5, 13-16, 26-36, 41-58%, for moisture, protein, carbohydrate, respectively and and 520-550 Kcal/100 g for energy). Local raw materials namely, Bambara groundnut and *Moringa oleifera* can be successfully used in the production of an alternative RUTF for severely acutely malnourished children and also as nutritious snacks for adults.

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KEYWORDS

Bambara groundnut, *Moringa oleifera*, malnutrition, ready-to-use therapeutic food, therapeutic food, protein concentrates, functional properties, plumpy'nut, and severe acute malnutrition

GLOSSARY

Terms/Acronyms/Abbreviation	Definition
BGN	Bambara groundnut
BGNF	Bambara groundnut flour
Functional properties	Inherent physical and chemical features that impacts protein performance in food systems during preparation, processing, manufacturing, and storage.
MO	*Moringa oleifera*
MOLP	*Moringa oleifera* leaf powder
Malnutrition	Refers to under nutrition or over nutrition
Nutritional properties	Nutritional value is the measure of the well-balanced ratio of the essential nutrients in food.
Protein concentrates	Used as functional ingredients to increase the nutritive value of foodstuffs
RUTF	Ready-to-use-therapeutic food
SAM	Severe Acute Malnutrition
Therapeutic food	Specially prepared energy and nutrient dense foods
TE	Total energy
UNICEF	The United Nations International Children's emergency fund
UNSCN	United Nations System Standing Committee on Nutrition
WFP	World Food Program
WHO	World Health Organisation

CHAPTER ONE
INTRODUCTION

1.1 Motivation of the study

Ready-to-use therapeutic foods (RUTF) are lipid-based, micronutrient enriched, and energy compact foods used in therapeutic feeding. They are produced in the form of a paste or crushable biscuit (Komrska 2007; Hassan *et al*., 2016). Principal ingredients for RUTF include peanuts, milk powder, vitamins, and mineral supplements, oil and sugar (Santini *et al*., 2013). The moisture content is low which makes them free from bacterial growth, hence enables outpatient care and can be used without refrigeration (Hassan *et al*., 2016). The nutritional profile of RUTF is similar to the therapeutic milk formula adopted for therapeutic feeding programs of inpatients in the facility centres. The protein requirement set by World Health Organisation (WHO) for RUTF is 10-12% of the total energy. It was developed in 1996, jointly by the French Institute of Research for Development and Nutriset (Komrska 2007; Weber *et al*., 2017). RUTF is used for the treatment of severe acute malnutrition without complication; SAM is the main killer of children between ages zero to five years, this account for about 1 million deaths annually. Approximately 20 million children are estimated to be suffering from this condition worldwide (Nyhq-asselin, 2013; Ismail & Suffla, 2013). RUTF is safe, cost-effective, and has saved the lives of many children when properly used, and it has been adopted by many countries as a part of the community-based management of SAM (Komrska, 2007). RUTF was approved as the worldwide standard of treatment for SAM by the World Health Organisation (WHO), UNICEF, the World Food Program (WFP), and the UN System Standing committee on nutrition (Komrska, 2007). Commercial forms of RUTF are Plumpy'Nut, a paste developed by Nutriset (France), Imunut by Diva (South Africa) and eeZeePaste by GC Rieber Compact. Although the product is well suited for the treatment of SAM, some of the ingredients used in its production are imported which makes the product expensive, this therefore minimises the product's affordability and availability. Nyhq-asselin (2013) reported that majority of RUTF is manufactured in and imported from the developed countries, local manufacturer, namely Diva SA, also imports some of the raw materials which are subject to import duties, whereas local possibilities exists to produce sustained improved nutrition for children. Two mainly expensive often imported raw material of RUTF are milk powder (Santini *et al.*, 2013), vitamin and mineral supplement. It is therefore essential to develop new formulae of RUTF with readily available local raw materials such as grains and legumes (Guimón & Guimón, 2015), this will enhance accessibility of the RUTF, as well as cost reduction. Bambara groundnut (BGN) and *Moringa oleifera* are examples of locally available legumes and plants, respectively that can be used in the production of RUTF.

Bambara groundnut (*Vigna subterranea*) (BGN) is a leguminous food crop (Bamshaiye *et al.*, 2011) that is indigenous to Africa. It yields a complete food because it contains

macronutrients and micronutrients, it is a good source of proteins, minerals and vitamins (Baryeh 2001; Kudre & Benjakul 2013). Macronutrient and micronutrient content of BGN includes; fat (6-9%), protein (19-21%), carbohydrate (55-70%), fibre (6.1-10.3%), ash (2.9-3.4%), calcium (0.03-0.098%), iron (0.007-0.009%), potassium (0.06-1.2%) and sodium (0.003-0.026%) (Ajayi & Lale 2000; Baryeh 2001; Steve Ijarotimi & Ruth Esho 2009; Eltayeb *et al*., 2011; Oyeyinka *et al*., 2015; Yao *et al*., 2015). The major minerals in BGN comprise phosphorous, magnesium, calcium while iron, copper, and zinc are the trace elements (Yao *et al*., 2015). The Bambara groundnut plant is highly drought tolerant and thus well adapted to climate changes (Oyeyinka *et al*., 2015). It has a high tolerance to poor soil, as well as the capacity to grow and produce in circumstances where peanut cannot survive (Bamshaiye *et al.*, 2011). The Bambara groundnut protein is high in lysine and methionine while tryptophan is the limiting amino acid (Abubakar & Dangambo, 2014; Yao *et al*., 2015). In comparison to soy protein, the protein isolate of Bambara groundnut is higher in lysine, arginine, and glutamic acid (Adebowale *et al*., 2007).

Moringa oleifera (MO) Lam belongs to the *Moringaceae* family, it is generally known as 'drumstick tree' or 'horseradish tree', (the drumstick describes the shape of its pods while horseradish describes the taste of its roots). MO plant grows rapidly and is expansively available in tropics and subtropics. It is economically significant to the food and medical industry (Makkar & Becker 1996; Richter *et al*., 2003). It is one of the most useful trees in the world because every part of the *Moringa* tree are essential for medication, food and industrial purposes (Moyo *et al*., 2011). MO leaves are rich in protein, iron, carotenoids, and ascorbic acid; and hence they serve as food and medicinal support for humans and are used as animal feed in the dry season (Makkar & Becker 1996; Richter *et al*., 2003; Anwar *et al*., 2007). Protein found in *Moringa oleifera* ranges from 16-40% (Elmoneim *et al*., 2007). Moyo *et al*. (2011) reported that 30.3% crude protein and 19 amino acids are present in the dried leaves of *Moringa oleifera*. The crude protein is close to that of sunflower cake seed, which is usually used as protein concentrates. Methionine and cysteine content in *Moringa oleifera* is very high and it is comparable to the content in human milk, cow's milk, and chicken egg (Oliveira *et al*., 1999). *Moringa oleifera* contains higher essential amino acids in comparison to the values specified for children below the age of 5 (Makkar & Becker, 1996).

Protein is an important component of cells and tissue and thus they are highly needed for the proper functioning of the body (Oloyede *et al.*, 2010). Bambara groundnut and *Moringa* are nutrient-dense foods, a good source of quality protein, and a rich source of minerals and vitamins. Complementing the protein of BGN and *Moringa* will provide a complete protein that can compete with other ingredients especially the milk powder used in the production of RUTF.

1.2 Statement of the Problem

Ready-to-use therapeutic foods (RUTF) have been confirmed operative in the management of severe acute malnutrition, but some ingredients needed for its production are not available locally. Plumpy'Nut, Imunut, and BP-100 are the commercial forms of RUTF. BGN and MO are nutrient-dense foods with quality protein that can be used as local raw material in the production of RUTF. They are both rich in amino acids, minerals, and vitamins. Complementing the protein of BGN with MO will provide a complete protein that will compete with other ingredients used in the production of RUTF and will meet the protein requirement (10-12% of the total energy) for RUTF set by WHO. However, the use of BGN and *Moringa* protein complex in RUTF is dependent on its nutritional and functional properties. These properties are inherent physical and chemical characteristics that impact the performance of protein in the food system during processing, manufacturing, storage, and preparation (Adebowale *et al*., 2007). There is therefore a need to examine the nutritional and functional properties of the BGN and MO protein complex and its application in a ready-to-use therapeutic bar.

1.3 The objective of the Research

1.3.1 Broad Objective

This study aims to investigate the nutritional and functional properties of the BGN and *Moringa* protein complex and its application in ready-to-use therapeutic food (RUTF).

1.3.2 Specific Objectives

The specific objectives include to:

1. Produce protein isolate from BGN flour and *Moringa* leaf powder.
2. Determine nutritional and functional characteristics of the BGN and *Moringa oleifera* protein isolates
3. Determine the nutritional and functional properties of the BGN-*Moringa* protein complex obtained from BGN and Moringa protein isolates.
4. Produce a ready-to-use therapeutic bar using the BGN-*Moringa* protein complex and profile its nutritional and functional properties.

1.4 Hypotheses

It is hypothesised that:

1. There will be differences between the nutritional and functional characteristics of BGN protein isolate and *Moringa* leaf protein isolate.
2. There will be differences in nutritional and functional properties of the BGN-*Moringa* protein complex compared to their isolates.
3. BGN-*Moringa* protein complex will be suitable to produce RUTF.

4. Ready-to-use therapeutic bar that will meet protein requirements for RUTF set by WHO will be produced.

1.5 Delineation

Undehulled BGN seeds as purchased and *Moringa oleifera* leaf powder will be used.

1.6 Significance of the Research

Knowledge of the application of BGN and *Moringa* protein complex in the production of a ready-to-use therapeutic bar for the management of SAM will be obtained. Food industry adoption of BGN and *Moringa* protein complex will lead to increase production of the complex. As well as increased production of BGN and *Moringa* ready-to-use therapeutic bar. Utilization of BGN & *Moringa* in the production of RUTF will lead to more consumption of BGN and *Moringa* resulting in improved nutrition and human well-being. More consumption will also lead to greater cultivation resulting in poverty alleviation and improved livelihood of the farmers.

Utilization of BGN & *Moringa* in RUTF will result in better availability of RUTF and increase the success story of community-based management of SAM in saving lives. Availability of the product will be enhanced because BGN and *Moringa* are locally available, difficulties and delays in the exportation of raw materials may be reduced.

The increased success story of community-based management of SAM by using BGN and *Moringa* ready-to-use therapeutic bar will fascinate the attention of NGOs and government organisations to sponsor the utilization of these local raw materials. Effective utilization of BGN and MO will prevent the extinction of the BGN seeds and MO plant, and thereby lead to economic, cultural, and diversity sustainability.

1.7 Expected Outcome, Result, and Contributions to Research

It is expected that:

1. BGN and MO protein concentrates will be produced.
2. Nutritional and functional characteristics of BGN and MO leaf powder will be established.
3. Nutritional and functional properties of the BGN and MO protein complex will be established.
4. Ready-to-use therapeutic bar that meets protein requirements for RUTF set by WHO will be produced.
5. A Master's in Food Science and Technology thesis will be produced
6. At least one scientific article will be published in an accredited journal.

1.8 Thesis Overview

This thesis consists of six chapters and was structured in article format where each chapter is an individual manuscript. The structure of the thesis is shown in Figure 1.1. Chapter one titled introduction gives the overview, which includes the research problem, objectives, hypothesis, delineations of the research, significance of the study as well as expected outcomes.

Chapter two is the literature review which expands further on the background of the research study. An overview of ready-to-use therapeutic food was reviewed, including the required standard for RUTF, production, and ingredients. Background of both Bambara groundnut and *Moringa oleifera* was outlined including utilisation, nutritional composition, and protein isolates from both legumes. Furthermore, an overview of protein was reviewed, including extraction of protein from the plant, optimisation of extraction methods, and physicochemical and nutritional characteristics of the protein.

Chapter three is the first research chapter focusing on the extraction of Bambara groundnut protein and *Moringa oleifera* leaf protein from Bambara groundnut flour and *Moringa oleifera* leaf powder, respectively. Phase behaviour of Bambara groundnut protein isolate and *Moringa oleifera* protein isolate in a solution was investigated through stability test, particle size analysis, and size distribution, particle dispersion stability, and syneresis test. An optimal mix for the two proteins was established.

Chapter four is the second research chapter focusing on the functional and nutritional properties of Bambara groundnut and *Moringa oleifera* leaf protein complex. Physical characteristics, functional properties, proximate, and amino acids of the individual protein isolates and their complex were determined.

Chapter five is the third research chapter focusing on the physicochemical and nutritional characteristics of a ready-to-use therapeutic food prepared from Bambara groundnut-*Moringa oleifera* leaf protein complex. Physical characteristics, nutritional, proximate, and sensory attributes of the produced ready-to-use therapeutic snack bar were assessed. Chapter six is the summary of the research including conclusions and recommendations.

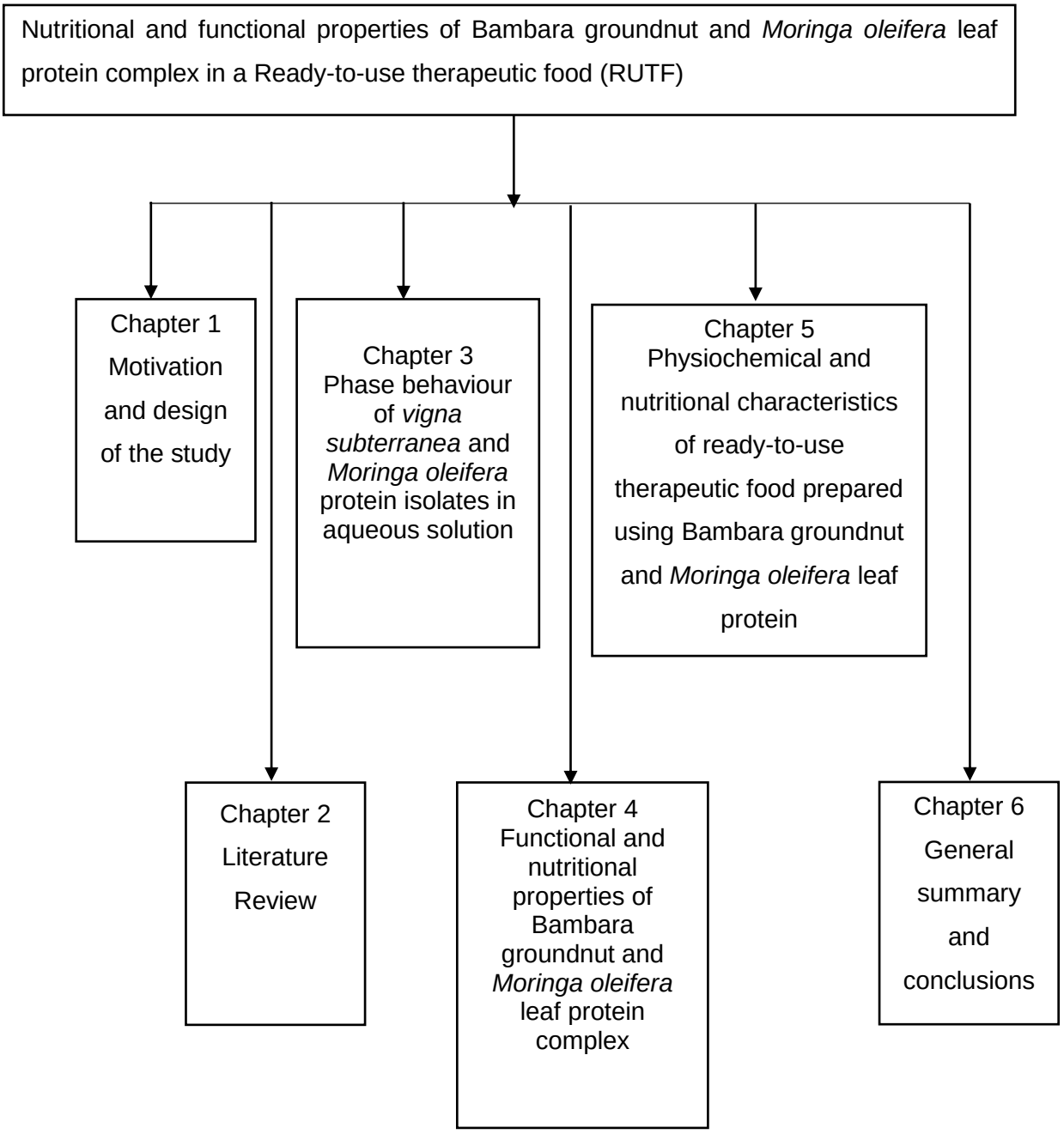

Figure 1.1: Thesis Overview

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CHAPTER TWO
LITERATURE REVIEW

2.1 Ready-To-Use Therapeutic Foods (RUTF)

Ready-To-Use Therapeutic Foods (RUTFs) are high in calorie, and rich in trace element. They are used in therapeutic feeding of children, and are produced as crushable biscuits or paste (Komrska 2007; Hassan *et al*., 2016). Milk powder, peanut, sugar, oil, vitamin and mineral supplements are the main ingredients used in the production of RUTF. They are mostly oil-based which makes them free from bacterial growth and enhances long shelf life without sophisticated packaging. It was developed in 1996, jointly by the French Institute of Research for Development and Nutriset (Komrska, 2007; Weber *et al*., 2017). The nutritional profile of RUTF is similar to the therapeutic milk formula (F100) used at the therapeutic feeding centres for the nutritional recovery of children suffering from severe acute malnutrition (SAM). RUTF is used for the treatment of SAM without complication, and there is no need for medical supervision. SAM is the main killer of children between ages zero to five years, this accounts for about 1 million deaths annually. Approximately 20 million children are estimated to be suffering from malnutrition worldwide (Nyhq-asselin, 2013; Ismail & Suffla 2013). Currently, 233.5 million children under the age of five are suffering from malnutrition worldwide (UNICEF/WHO/THE WORLD BANK, 2021). RUTF is cost-effective and lives of many children has been preserved due to its proper usage.

RUTF has been adopted by many countries as a part of the community-based management of SAM (Komrska, 2007). RUTF was authorized by UNICEF, the World Health Organisation (WHO), the World Food Program (WFP), and the UN System Standing Committee on nutrition as the standard for the management of SAM worldwide (Komrska, 2007). Commercial forms of RUTF are Plumpy'Nut, a paste developed by Nutriset (France), BP100 (a biscuit produced from cooked wheat) developed by GC Rieber Compact (Denmark), and Imunut by Diva (South Africa).

Although these products are very suitable in the treatment of SAM, some of the ingredients (Milk powder, vitamin, and mineral) are imported from developed countries and thus make the final product expensive, thereby affordability and availability of the product are minimized (Santini *et al.*, 2013). Whereas local possibilities exist to produce RUTF from local sources here in South Africa (Nyhq-asselin, 2013). It is therefore essential to develop innovative formulations of RUTF using cereals and legumes that are available locally (Guimón & Guimón, 2015), to enhance the accessibility and affordability of the RUTF. Bambara groundnut (BGN) and *Moringa oleifera* leaf powder are examples of locally available legumes that can be used in the production of RUTF.

2.1.1 RUTF Standard

RUTF must be made according to the standard defined by UNICEF, WHO, WFP, and the UNSCN. RUTF should be high-energy food and fortified with vitamins and minerals. It should be suitable for treatment of severely malnourished children and should be ready to consume. It should be Indulgent, delicious, and easy for children to consume without any need of preparation. Fifty percent of the protein contained in the product should be from milk products and a shelf life of two years. Nutritional specifications are shown in Table 2.1.

2.1.2 RUTF Production and Ingredients

Ingredients used in the production of RUTF are peanut butter, vegetable oil, full-fat milk powder, sugar, vitamins, and minerals. There is no need to add water to the formulation, this result in a product with low moisture content and low water activity, which prevents the proliferation of bacteria, and thus shelf-stable product. RUTF is nutritionally equivalent to F100 (a milk-based-therapeutic diet used for patients in phase 2) for home uses. It was prepared in the form of semi-solid or solid and its nutrient profile is comparable to F100. RUTF can be produced either locally or abroad. Some countries in Africa and Asia have successfully produced RUTF in their communities using local raw materials. These countries include; Ethiopia, Malawi, Niger, the Democratic Republic of Congo, Sri Lanka, Indonesia, and Pakistan (Schoonees *et al.*, 2013). Ingredients that have been used in the local production of RUTF are legumes (groundnut, almond, lentil, and soybean), cereal/grain (maize and oat), milk (acid whey, non-fat dry, and whey protein concentrate), oil (canola rapeseed, coconut, palm, soybean, and sunflower), sugar and cocoa (Weber *et al.*, 2017).

RUTF provides a balanced, nutritious, and home-based therapy. It provides better affordability compared to facility-based care. There is no need to mix RUTF with water because it is ready to use. It is safe for consumption at home without refrigeration and even in places where hygienic conditions are not very good. The product has extended shelf life. There is no need for any form of preparation before consumption, it can be consumed straight from the packaging (Schoonees *et al.*, 2013; Hassan *et al.*, 2016).

Table 2.1: Specifications for RUTF1

	Composition	
Nutrient	**Min**	**Max**
Moisture content (%)	-	2.5
Energy (Kcal/100g)	520	550
Proteins (%TE)	10	12
Lipids (%TE)	45	60
Sodium (mg/100g)	-	290
Potassium (mg/100g)	1100	1400
Calcium (mg/100g)	300	600
Phosphorus (excluding phytate) (mg/100g)	300	600
Magnesium (mg/100g)	80	140
Iron (mg/100g)	10	14
Zinc (mg/100g)	11	14
Copper (mg/100g)	1.4	1.8
Selenium (µg)	20	40
Iodine (µg/100g)	70	140
Vitamin A (mg/100g)	0.8	1.1
Vitamin D µg/100g	15	20
Vitamin E (mg/100g)	20	-
Vitamin K µg/100g	15	30
Vitamin B1 (mg/100g)	0.5	-
Vitamin B2 (mg/100g)	1.6	-
Vitamin C (mg/100g)	50	-
Vitamin B6 (mg/100g)	0.6	-
Vitamin B12 (µg/100g)	1.6	-
Folic acid (µg/100g)	200	-
Niacin (mg/100g)	5	-
Pantothenic acid(mg/100g)	3	-
Biotin (µg/100g)	60	-
n-6 fatty acids (% TE)	3	10
n-3 fatty acids (%TE)	0.3	2.5

[1] WHO/WFP/UNSCN/Unicef, 2007. *TE = Total energy

2.1.3 Nutrition Issue of children below the age of five years

Children living in underdeveloped countries are most likely at risk of malnutrition in the early years of life, this age group has a high demand for dietary requirements (Guimón & Guimón,

2015). This is an important stage of growth and development; hence, a proper diet is not negotiable. Malnutrition accounts for more than one-third of total child deaths, even though it is seldom listed as the direct cause (Nabuuma *et al.*, 2013). Causes of malnutrition are inaccessibility to nourishing food, insufficient breastfeeding, and an unbalanced diet (Nyhq--Asselin, 2013). Additionally, infections such as recurrent diarrhoea, measles, malaria, and pneumonia, are major challenges to a child's nutritional status, which consequently puts the child at risk of impaired growth.

Severe acute malnutrition is a condition-affecting infant below the age of five years in many developing countries and it is linked with morbidity and death. It is characterized by weight loss in comparison to height, noticeable austere wasting, and/or the incidence of oedema. About 20 million children were reported to be suffering from SAM worldwide in 2013 (Nyhq-asselin, 2013; Ismail & Suffla 2013). The statistics can be frightening and disheartening, however, SAM can be prevented and successfully treated with RUTF.

High-energy milk (F-75 and F-100) are therapeutic powdered milk used in the management of SAM. They can only be used in therapeutic feeding centres (TFCs) where close supervision of preparation and utilization are ensured. The therapeutic powdered milk has to be diluted with potable water (preferably boiled water) and consumed within a limited period to restrict bacterial growth. F-75 is the 'starter' formula given to infants whose bodies are not able to tolerate the regular nutrient and are hospitalized. It is also known as phase 1 or the stabilization phase. F-100 is a milk based-therapeutic diet and called phase 2. It is also known as the 'catch-up' because it is given to commence weight gain (Schoonees *et al.*, 2013). Therapeutic foods are specially prepared food products that supply complete nutrients. They are designed to be used in emergencies as medicine or supplements for the treatment of SAM in children (Hassan *et al.*, 2016). Bambara groundnut (BGN) and *Moringa oleifera* leaf powder are examples of nutrient-rich legumes that can be used in the production of RUTF.

2.2 Bambara Groundnut

Bambara groundnut (*Vigna subterranea*) is a leguminous plant that has great potential of becoming an industrial food raw material for food security and alleviation of poverty in Africa, although it is considered as one of the industrially under-utilized crops. BGN belongs to the family *Fabaceae*; botanically it is known to have two varieties namely *Vigna subterranean* var. *spontanea* (the wild varieties) and *Vigna subterranean* var *subterranean* (the cultivated varieties) (Yao *et al.*, 2015). It is comparable to cowpea and peas in cultivation (Oyeyinka *et al.*, 2015). After groundnut and cowpea, Bambara groundnut (BGN) is the third most important legume in many African countries (Adegbola & Bamishaiye, 2011; Hillocks *et al.*, 2012, Oyeyinka *et al.*, 2015). BGN is prevalent in Africa and it is identified with several names with different local languages, but in literature, Bambara groundnut is the preferred name, which is derived from a tribe called 'Bambara' in Mali. The various names and the associated languages

are shown in Table 2.2. The nuts were originally found in the Sahelian region of present-day West Africa, (Adegbola & Bamishaiye, 2011). They are now widely cultivated in many parts of Africa and tropical regions, they can be found in many parts of South America, Asia, and Oceania (Baudoin & Mergeai, 2001). Previously, was mostly grown by the rural dwellers for home consumption in South Africa but is now being sold in the markets as raw bean seeds and as boiled groundnut. In South Africa, it is mostly grown in three provinces, which are Kwazulu Natal, Mpumalanga, and Limpopo provinces.

Table 2.2 Local Names of Bambara Groundnut in Different Countries[1]

Countries	**Name**
South Africa	*Jugo, Indlubu, Jugoboo, Ndhu nwa tzidimba*
Republic of Zambia	*Ntoyo ciBemba*
Nigeria	*Gurjiya or Kwaruru, Okpa, Epa-Roro*
Zimbabwe	*Nyimo, Indlubu*
Akan tribes of Côte d'Ivoire,	*Clô-Nglô*
Botswana	*Ditloo*
Ghana	*Azi nogui*
Kenya	*Njugu mawe* (Swahili)
Centra Africa	*Njogo* bean
Indonesia	*Kacangbogor* (bogor peanut)
DRC	Congo groundnut, Congo *goober*
Madagascar	*Pistache, Voanjobory*
Malawi	*Nzama, Njama, Voandzounzama*
Sierra Leone	*Agbaroro*
Swaziland	*Tindhluwa*

[1](Bamishaiye *et al*, 2011; Hillocks *et al.*, 2012)

The Bambara groundnut plant is drought tolerant and able to adapt to climate changes (Oyeyinka *et al.*, 2015). It has a high tolerance for poor soil and can yield in circumstances that are hostile to the growth of peanut (Bamshaiye *et al.*, 2011). Bambara groundnut can resist drought conditions better than other legumes and has been reported to grow well when rainfall is low (Bamshaiye *et al.*, 2011; Hillocks *et al.*, 2012). The seed coat of BGN is tough which makes it resistant to pests and disease. The tough seed coat enhances its tolerance to weevil invasion and as such storage without major damage is guaranteed (Collision *et al*., 2000).

In many African countries, BGN is grown mostly by women and their farming activity is on a small scale. BGN is cultivated as a sole crop and it is also planted among other crops such as maize, cowpeas, and melon (Bamshaiye *et al.*, 2011). Bambara groundnut is an annual, intermediate, and non-woody plant that supports land care provision in Africa based on the root nodules (National Research Council, 2006), and hence possess great prospective to alleviate food scarcity and poor nutrition. Bambara groundnut seed is housed in a hard seed coat; it is smooth and usually round in shape, the size varies in diameter of about 1.5 cm (Bamshaiye *et al.*, 2011).

The seed comprises adequate amounts of macromolecules such as protein, carbohydrate, and fat, thus provides wholesome food. It is conservatively categorized as beans even though it is cultivated from the ground in the same manner as peanuts. The pods are rounder and larger than peanut shells and the seeds inside are shaped more like peas than peanuts. They are round in shape, tasty and nutritious. They exist in different colours and patterns as shown in Figure 2.1, which makes them very attractive. They are categorized by beautiful local names such as dove eyes, nightjar, and butterfly (National Research Council, 2006). The seed differs in shape, colour, and size of the outer seed coat. They may be round or elliptical with different colours such as cream, brown, red, black, the weight of seed are usually within 280 and 320 g (Bamshaiye *et al.*, 2011).

2.2.1 The utilisation of Bambara groundnut

Bambara groundnut plays a significant role and is extensively used in traditional dishes in African countries. The seed is prepared and eaten in different ways, freshly harvested seeds can be eaten raw; the seeds can as well be dried and stored. The stored seeds are however soaked and boiled before consumption. The dry seeds can be processed into a powder to prepare cakes (Adebowale & Lawal, 2002).

In Nigeria, BGN is utilised in many ways; it can be roasted or boiled and eaten as snacks, or milled into flour and used in the preparation of porridge, ‘tuwo’, soup steamed food like ‘okpa’, ‘moin-moin’, or fried as ‘akara’. It can also be used in the production of local beverages such as ‘kunu’ (Adeleke *et a*l 2017). BGN is also used for medical purposes in North-Eastern Nigeria (Atiku & Mohammed (2004). Pirate (2008) reported that the flavour of milk prepared from BGN is preferred in flavour when compared with soybean, cowpea and pigeon pea milk.

Figure 2.1 Bambara Groundnut Seed [Source: Jideani, (2015)]

2.2.2 Nutritional Composition of Bambara groundnut

Bambara groundnut (BGN) is a complete food because it is nutrient-dense. It could therefore be used to solve malnutrition and also increase food security (Yao *et al.*, 2015). It is rich in proteins, carbohydrates, fat and contains a certain amount of minerals and vitamins (Baryeh 2001; Adegbola & Bamishaiye, 2011; Kudre & Benjakul 2013). BGN is very nutritious and the seeds contain 17-25% protein, 55-70% carbohydrate, 6-9% fat, 6.1-10.3% fibre, 2.9-4.4% ash, 0.03-0.097% calcium, 0.007-0.009% iron, 0.06-1.2% potassium and & 0.003-0.026% sodium (Ajayi & Lale 2000; Baryeh 2001; Sirivongpaisal, 2008; Steve Ijarotimi & Ruth Esho 2009; Eltayeb *et al*., 2011; Oyeyinka *et al*., 2015; Yao *et al*., 2015). Major minerals in BGN are phosphorous, magnesium, calcium, while iron, copper, and zinc are trace elements (Yao *et al.*, 2015). BGN is richer in essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine) than peanut (Bamshaiye *et al.*, 2011). Linoleic, palmitic, and linolenic acids are the main fatty acids in BGN (Minka & Bruneteau, 2000). The carbohydrate component is made of starch and non-starch polysaccharides, as well as a little amount of reducing and non–reducing sugars (Adegbola & Bamishaiye, 2011).

2.2.3 Bambara groundnut protein isolates or concentrates

The BGN contains 85.97-91.00% protein, 3.2% fat, 3.11% ash, moisture 3.16 moisture, and 5.48 carbohydrate. Its protein contains all the essential amino acids, however, tryptophan is said to be the limiting amino acid (Yao *et al*., 2015) and is high in lysine and methionine (Abubakar & Dangambo, 2014). The total amino acid varies between 81.07-90 g/100g (Adebowale *et al.*, 2011; Eltayeb *et al.*, 2011; Kudre *et al.*, 2013a; Arise *et al.*, 2015). Several authors reported Bambara protein comparable to recommended guidelines by FAO/WHO/UNU (Adebowale *et al.*, 2011; Kudre *et al.*, 2013a; Arise *et al.*, 2015). Extraction methods for BGN include alkaline extraction/isoelectric precipitation, micellar precipitation, air classification, salt extraction-dialysis, water extraction, and ultrafiltration (Adebowale et al., 2011; Boye *et al.*, 2010; Oujifard *et al.*, 2012; Kudre *et al.*, 2013; Stone *et al.*, 2015). The starting material is a major determinant of the differences in results obtained by different authors, some studies were with whole flour (Oujifard *et al.*, 2012; Kudre *et al.*, 2013) while others with a specific variety of the flour (Adebowale *et al.*, 2011) such as brown or white.

2.3 Moringa oleifera leaf powder

Moringa oleifera (MO) Lam belongs to the *Moringaceae* family, it is known as ‘drumstick tree’ or ‘horseradish tree. The name ‘drumstick tree’ describes how its pod is shaped and ‘horseradish tree’ describes the taste of its roots (Anwar *et al.*, 2007). MO tree ranges from 5-10 m in height (Anwar *et al.*, 2007). It is indigenous to North-West India, and it has become an important plant in many parts of Asia, Africa, and America due to its abundant economic significance for the food and medical industry. It is mostly planted in hedges and home yards (Perumal & Klaus 2003; Anwar *et al.*, 2007). It is a fast-growing plant, and it is usually accessible in tropics and subtropics.

2.3.1 Utilisation of *Moringa oleifera* leaf powder

Moringa oleifera is one of the top valuable trees in the world. Different part of MO tree can provide useful ingredients for food and medication, and raw materials for industrial purposes (Moyo *et al.*, 2011). The leaves, fruits, flowering parts, and unripe pods of MO are utilised as vegetables in several parts of Africa, India, Pakistan, Philippines, and Hawaii; and they are very nutritive (Perumal & Klaus 2003; Anwar *et al.*, 2007). The extract from different parts of MOLP are used for various purposes; root (for spices); seed (for cooking and cosmetics oil); all plant organs (medicinal use) (Bukar *et al.*, 2010; EL-Massry *et al*., 2013) Aqueous extract from raw seeds of *Moringa oleifera* has a flocculating protein that is used as a purifying agent in water purification (EL-Massry et al., 2013). MO leaf paste is used as an external application for wounds (Perumal & Klaus 2003). Extract from the leaves is used as an oil, curry powder, and in water purification (Bichi M., 2013). It has been used in the formulation of infant food to

increase protein content (Anwar *et al.*, 2007). The boiled leaves of *Moringa* are used to feed babies in the Philippines.

2.3.2 Nutritional Composition of *Moringa oleifera* leaf powder

Moringa oleifera (MO) leaves are a good source of protein. MO leaves are also a good source of carotenoids, ascorbic acid, calcium, potassium, and iron. The leaves are identified as a food source for human beings and animal feed especially during the dry season (Richter *et al*., 2003; Anwar *et al.*, 2007). Protein found in MO fresh leaves was reported to be 16.7% (Elmoneim *et al*., 2007), while the dried leaves of MO contain 30.3% crude protein and 19 amino acids (Moyo *et al*., 2011). The crude protein value is close to the crude protein of sunflower cake seed which is usually used as protein concentrates. MO leaves are a rich source of tryptophan, methionine, lysine, and cystine, (Perumal & Klaus, 2003). Methionine and cysteine in *Moringa* seed are very high and it is comparable to that of human milk, cow's milk, and chicken egg (Alli *et al.*, 2017). The essential amino acid in *Moringa* olefera leaves contains higher essential amino acid in comparison to soya bean and fulfils the WHO requirement (Koul and Chase, 2015).

2.3.3 *Moringa oleifera* leaf protein concentrate

Moringa oleifera leaf protein concentrate (MOLPC) is highly nutritive containing 38.02-39.13% protein, 2.43-3.2% fat, 6-9.02% ash, fibre 5.43-13.94% fibre, 5.92 moisture, and 38.21% CHO. MOLPI contains substancial amounts of micronutrients like magnesium, zinc, sodium, potassium, calcium, phosphorous, iron, manganese, and copper as shown in Table 2.3 (Sodamade and Adeboye, 2013). Isolates are used as functional ingredients to increase the nutritional quality of food products (Wani *et al.*, 2015). Methods used in the extraction of protein concentrates from MO include; ethanol extraction, methanol extraction, alkaline extraction/precipitation, water extraction, and heat treatment (Sodamade and Adeboye, 2013; Ahmed, 2016).

Table 2.3 Mineral composition of *Moringa oleifera* protein concentrates

Micronutrients	**Concentration mg/100g**
Potassium	23.20
Sodium	214.00
Calcium	723.00
Magnesium	677.00
Phosphorous	5.00
Iron	187.00
Manganese	252.00
Copper	55.00
Zinc	548.00

Sodamade & Adeboye, 2013.

2.4 Overview of Proteins

Proteins are polymers of amino acids, each protein-polymer is referred to as polypeptide, which consists of a sequence of different L-α amino acids. An α-amino acid consists of a central carbon called the α carbon linked to an amino group, a carboxylic group, a hydrogen atom, and a distinctive R group. Two mirror forms of amino acid are L and D isomers. Only L amino acids are found in proteins. The twenty amino acids that make up a protein molecule include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine. Protein performs a great variety of roles in all organisms which make them indispensable and their performance is enhanced by the adaptability of the inbuilt amino acids (Moyo *et al.*, 2011). They are the ultimate micro-machines with multiple functions. Some are building blocks which joins with other substances to make the cells from which we were formed; some are catalysts that speeds up biochemical reactions to keep our cells active and alive; while others assists the cells in carrying out functions such as movement, communication, building of body tissue, and regulation of unfolding programs for development. Their enormous functions and link to life make them very relevant in food processing. Moreover, proteins possess physicochemical and nutritional properties, which enhance their significance in food product development and processing. The two major sources of protein are animal and plant sources. Animal protein consists of high fat and can lead to diseases such as high blood pressure, and heart disease when consumed in large quantity and are also expensive. Plant protein is cheaper and have a lower level of disease risk, but are lower in some amino acids compared with animal protein (Nabuuma *et al.*, 2013). However, plant protein can be used in conjunction with cereals to provide a complete protein.

There is an increasing challenge in accessing animal protein such as milk and gelatin products in developing countries due to economic reasons and an increase in population leading to malnutrition, especially in growing children. Therefore there is a need for an inexpensive and sustainable source of protein, hence, it is necessary to search for alternatives sources of protein (Adebowale *et al.*, 2011; Sodamade and Adeboye, 2013). Legumes and vegetable plant leaves have been reported as an alternative source of protein for humans. Legumes contain 20-50% protein which is higher than protein in root crops and twice higher than the level in cereal grains. Their protein is usually extracted into concentrated protein powder either as isolates or concentrates, which offers a huge opportunity in new product development of food products (Eltayeb *et al.*, 2011).

Protein concentrates contain a significant amount of carbohydrates and fat, but protein isolates have a very low level of those macromolecules. Concentrated protein powders from legumes and plant leaves are produced through various extraction methods. Leaf protein is a good source of essential amino acids, methionine is the limiting amino acid. Leaf protein concentrates have a higher nutritive value than soybean meal when properly processed. Legume concentrate or isolates may contribute majorly to health by lowering plasma cholesterol, prevent cancer, diabetes, and obesity, and protecting against bowel and kidney disease (Kudre *et al.*, 2013). They contain a substantial amount of amino acids, which are categorized as essential and non-essential and differ according to animal species. They are indispensable in the production of enzymes, immunoglobins, hormones, growth, and repair of body tissues and form the structure of red blood cells (Brisibe *et al*., 2009). Furthermore, they play an important role in the formation of glucose, acting as a buffer when other precursors are in short supply. Each amino acid has a specific function in the animal's body.

2.4.1 Extraction of protein from plant

The aim of extracting protein is to obtain concentrated forms either as protein isolates or concentrates which are used for the improvement of nutritional and functional quality of food (Adebowale *et al.*, 2011). Some known methods of extracting protein include alkaline extraction/isoelectric precipitation (AE-IP), salt extraction-dialysis, air classification, ultrafiltration, and heat extraction as shown in Table 2.4.

Alkaline extraction/isoelectric precipitation: Aqueous alkaline extraction followed by isoelectric precipitation (IEP) is a technique that takes advantage of the solubility of legume protein. The solubility is high at alkaline pH and low at pH values close to their isoelectric point. It is the most commonly used for protein extraction. Many researchers have extracted protein from plant material such as soya bean, chickpea, broad bean, kidney bean, BGN, and others using this procedure (Adebowale *et al.*, 2007, 2011; Zare *et al.*, 2010; Oujifard *et al.*, 2012; Kudre *et al.*, 2013).

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Table 2.4 Extraction methods for Protein concentrate and Isolates

Flour	Protein/Concentrate	Extraction Method	References
BGN flour	Protein Isolate	Alkaline extraction/isoelectric precipitation Micellisation technique Air classification	Yemisi *et al*., 2011, Oujifard *et al*., 2012 Diedericks *et al.*, 2020
Mucuna Beans flour	Protein isolate	Alkaline extraction / isoelectric precipitation	Adebowale *et al*., 2007
Pea flour	Protein isolate	Alkaline extraction / Isoelectric precipitation Salt extraction-dialysis Micellar precipitation:	Stone *et al*., 2015.
Mung bean, black bean and BGN	Protein isolate	Alkaline extraction / Isoelectric precipitation	Kudre *et al*., 2013
Pea, chickpea, and lentil flour	Protein concentrate	Ultrafiltration Alkaline extraction / Isoelectric precipitation	Boye *et al.* (2010).
Cassava leaf flour	Protein concentrate	Heat application	Fasuyi & Aletor, 2005; Aletor, 2010.

BGN: Bambara groundnut flour.

Salt extraction-dialysis: This is based on the salting-in and salting-out phenomenon of food proteins (Zare *et al.*, 2010). The salt solution at the desired ionic strength is used to extract the protein. Ammonium sulfate is the most commonly used precipitant for salting out of proteins. At saturation, it precipitates most proteins and protects proteins in solution from denaturation and bacterial growth. The protein solution is thereafter diluted to induce protein precipitation. The precipitated protein is usually recovered by centrifugation or filtration, followed by dialysis with distilled water. The obtained product is then freeze-dried. The dialysis technique is used for removing salt and other small molecules from the protein mixture. It involves the use of a semipermeable membrane with pores. This technique has been used in extracting protein from pea flour and BGN flour (Arise *et al.*, 2015; Stone *et al.*, 2015).

Micellization: This method involves precipitation with a neutral salt by dilution in cold water. NaCl is mostly used, the produced protein has a micellar structure before drying. Stabilization of the isolate is enhanced by hydrophobic interactions (Rodríguez-Ambriz *et al.*, 2005; Adebowale *et al.*, 2011; Arise *et al.*, 2015; Stone *et al.*, 2015; Hadnađev *et al.*, 2018).

Air-classification: Air classification is a dry processing method in which the sample is separated into starch and protein fractions by milling and air classification. The milling process results in the production of flour which is different in size and density, fine and heavy coarse fraction. Air current is used to separate the two phases, which is the origin of the name air classification. The fine and lighter particles are mainly starch and fibre while the coarse and heavier particles are protein and lipids. To obtain pure fractions, air classification is repeated which is a drawback due to loss of product. It is more efficient on products with starch as their main storage material namely pea, faba bean, cowpea, lima bean, and BGN (Zare *et al.*, 2010; Diedericks *et al.*, 2020a).

Heat extraction: When proteins are heated above a certain temperature, they are denatured and separated from the solution. Protein concentrates of common leafy vegetables and cassava leaves were coagulated by heating between 80-90^{o}C ((Aletor *et al.*, 2002; Fasuyi & Aletor, 2005; Aletor, 2010).

2.4.2 Optimisation of protein extraction methods

A method of extracting protein isolate from three pea cultivars was optimised by Stone *et al.* (2015). The study compared 3 extraction methods: 1) alkali extraction/isoelectric precipitation (AE-IP) at pH 9.50 using 1.0 M NaOH and pH 4.50 with 1.0 M HCL, 2) salt extraction-dialysis (SE) with 0.1 M sodium phosphate buffer, 3) micellar precipitation (MP) with 1.0 M NaCl. Salt-extraction-dialysis gave the highest extraction yield followed by AE-IP, while MP gave the least. The extraction of protein isolate from two varieties of Bambara groundnut was optimised. The protein isolate was extracted using alkaline extraction/isoelectric precipitation (AE-IP) and micellisation (Adebowale *et al.*, 2011). The isolate produced by AE-IP gave a high yield while the micellised isolate had better functional properties. Extraction of protein isolate from *Lupinus*

campestris was carried out by Rodríguez-Ambriz *et al.* (2005). The study compared 2 methods: 1) isoelectric precipitation, 2) micellisation. The researcher reported higher protein recovery for isoelectric precipitation. Protein concentrate from BGN was extracted using 1) acid precipitation at pH 8.0 with 1 M NaOH, and pH 4.0 with 0.5 M HCL, 2) salt solubilisation with 0.5 M NaCl (Arise *et al.*, 2015). The researcher reported higher yield with acid precipitation and higher functional properties with salt solubilisation.

2.4.3 Physiochemical and nutritional characteristics of proteins

The physical and chemical properties of proteins are inherent attributes that impact the performance of protein during the handling, manufacturing, storage, and preparation of food (Adebowale *et al.*, 2007). Gelation, emulsion capacity (activities and stability), water and oil absorption capacity, foam capacity and stability, protein solubility, and bulk density are some of the functional property requirements of protein. All these characteristics contribute significantly to the physical and chemical attributes of the food systems they are used in.

Gelation: Least gelation concentration (LGC) is the index of gelation capacity. The lowest protein concentration is described as the concentration at which a test sample (gel) did not slip from the inverted tube. The gelation ability of protein is determined by LGC. Gelation ability of protein ingredients is best at lower LGC (Eltayeb *et al.*, 2011).

Emulsion capacity (activities and stability): Emulsification activities of protein-containing products are enhanced by the availability of soluble and insoluble protein, and polysaccharides needed to form and stabilize an emulsion. The emulsion stability of a protein is established by its ability to impart strength to an emulsion by reducing the superficial pressure and increasing electrostatic repulsion on the superficial of the oil droplet (Sikorski, 2002). The viscosity of the system is increased by the addition of polysaccharides which provide additional support for the stabilization of the emulsion (Melanie *et al.*, 2020).

Foam capacity and stability: Foam capacity is the capability of a solution to produce stable foam. Foam is produced when air is whipped into a liquid as quickly as possible (Sikorski, 2002). The proteins in flours are surface-active, hence flour can produce foams. However, stabilization of the foam is made possible due to the interaction of protein. Increased flexibility exists at the air-liquid boundary as protein molecules unfold and interact with one another to form multilayer protein film, thereby prevents the breakage of air bubbles, and the foam is thus stabilized. Surface tension at the boundary between air bubbles and surrounded liquid is further reduced by soluble protein, thereby obstructing coalescence of the bubbles and stabilizing the foam (Adebowale & Lawal, 2003).

Protein Solubility: Solubility is an important test in the investigation of the functionality of a protein. Solubility can be influenced by external and internal factors. External factors that can influence the solubility of protein include; pH, ionic strength, temperature, and the presence of various solvent additives (Kramer *et al.*, 2012).

Water Absorption Capacity (WAC): WAC is important in food processing; it impacts the viscosity, bulking, and consistency of products. It is also crucial in the application such as baking application (Niba *et al*., 2001). Water plays a major role in chemical reactions that occur during mixing and baking. It is therefore essential to understand the chemical nature and properties of all components involved and be able to determine their WAC (Guzmán *et al*., 2015). Proteins and carbohydrates are the main chemical constituents that enhance the WAC of flours (Lawal and Adebowale, 2004).

The oil absorption index (OAI) is important because oil plays a significant role in the food system. Oil functions as a flavour retainer, mouth feel enhancer, taste improver, and shelf-life extender of food products especially in processes where fat absorptions are required (Aremu *et al*., 2007). Hydrophobic proteins show superior binding to lipids.

Bulk Density (BD): BD is a physical property that affects the structural loads of the sample, it is a significant factor in drying and storing structure (Mpotokwane *et al.*, 2008). BD depends on the collective effects of interconnected factors namely; particle size, the intensity of attractive inters particle forces, and several contact points (Wani *et al.*, 2015). Bulk density is influenced by starch polymer structure and that low BD could be a result of loose starch polymer (Adeleke *et al.*, 2018). Swelling capacities are dependent on the nature of materials, type of treatment, and process conditions and it is influenced by temperature changes. The swelling capacity of flour generally increased with temperature (Adeleke *et al.*, 2018)

Industry and protein isolate usage are shown in Table.2.5.

Table 2.5 Different properties of protein isolate that can be used in food products

Industry	**Product**	**Property of protein isolate**	**Source**
Beverage	Drink	Nutritional additive	Stone *et al.* (2015)
Bakery	Bread	Nutritional additive	Stone *et al.* (2015)
	Cake	Increase shelf life	
Dairy	Ice cream	Reduce syneresis	Adebowale *et al.* (2011)
	Yoghurt		
Sauce	Sauces	Thickener	Adebowale *et al.* (2011). Stone *et al.* (2015)
Processed meat	Sausage	Emulsion stabilizer Increase shelf life	Adebowale *et al.* (2011)

2.5 Conclusions

Ready-to-use therapeutic foods (RUTF) are suitable in the treatment of severe acute malnutrition, however, production of an alternative RUTF from legumes is essential to enhance affordability and availability of the product. Bambara groundnut (BGN) and *Moringa oleifera* leaf powder (MOLPI) are nutrient-dense legumes and are considered complete foods. Notably, is the high content of protein in these legumes and they are also a good source of vitamin and mineral. Their protein isolates may be used as a replacement for the full-fat milk powder in ready-to-use therapeutic foods and as functional ingredients in other food products.

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Verdc. *(Fabaceae)]* produced in Côte d'Ivoire. *International Journal of Molecular Sciences*, **16**, 21428–21441.

CHAPTER THREE

PHASE BEHAVIOUR OF *VIGNA SUBTERRANEA* AND *MORINGA OLEIFERA* PROTEIN ISOLATES IN AQUEOUS SOLUTION

ABSTRACT

Phase behaviour of Bambara groundnut protein isolate (BGNPI) and *Moringa oleifera* protein isolate (MOLPI) was investigated to study their interaction and stability in a water system. Bambara groundnut seeds were dried at 50^{o}C for 48 hours and milled into flour. The flour was defatted with 80% ethanol and subjected to isoelectric precipitation under heat and no heat application to obtain BGNPI. Protein isolate of *Moringa oleifera* leaf powder was obtained by multiple methods namely; metabisulphite (2%) in sodium citrate buffer at pH 8, methabisulphite (2%) in sodium citrate buffer at pH 6, solubility and precipitation at pH 7, solubility and precipitation at pH 12, sonication, homogenization and heat method at 90^{o}C. The protein content of BGNPI ranged from 61.1 to 83.2% and MOLPI from 30.3 to 57.7% and their yield on protein base ranged from 45.8 to 95.36% and 3.7 to 65.7%, respectively. BGNPI (1, 5, 9% w/v) and MOLPI (5 to 4.5% w/v) were varied at two levels each based on a 2^2 factorial experimental design augmented with centre points as independent variables in 100% distilled water. Eleven (11) compositions were established, and the effects were determined on the equilibrium backscattering (BS) flux as the stability indicator for the protein mixture. Sedimentation, creaming, and flocculation was the destabilisation mechanism of the protein mixture. The protein mixture was stable to creaming and sedimentation at a higher concentration of BGNPI and MOLPI. A zetasizer Malvern Instruments (NanoZS) was used to study particle size, and zeta potential and syneresis were investigated by visual observation. Numerical optimisation was used to determine the most stable composition of the protein mixtures based stability index, syneresis (%), and particle size. A protein mixture with 9% BGNPI and 2% MOLPI was established as the optimal mix for the protein isolates.

3.1 Introduction

Proteins are used as food ingredients due to their vital importance in the human diet (Adebowale *et al.*, 2007). They are also important in food product development and processing due to their functional properties that impact consumer acceptability including physicochemical and nutritional properties. Nevertheless, protein malnutrition is a major nutritional problem, especially in the developing world (Ijarotimi *et al.*, 2009). Mostly, there are two main sources of protein which are either animals or plants. Protein from animal sources is expensive and not affordable for most of the population, it is also linked to health issues. Protein from animal origin includes whey protein, gelatin sodium caseinate, milk, and eggs. Consequently, there has been a constant search for alternative protein sources to meet human nutrition

requirements. Therefore, research focus has been on the use of plant protein in the formulation of new food products to enhance adequate supplies of food protein (Taherian *et al.*, 2011). Proteins from a wide range of plant sources are broadly used in human nutrition in the forms of isolate and concentrate.

Protein isolates or concentrates are sources of beneficial and active compounds, especially amino acids; hence they are used as functional ingredients to increase the nutritional quality of food products (Wani *et al.*, 2015). Protein isolate is a more purified form of protein, most of the non-protein components had been removed. Protein isolate is about 90% pure protein with a natural flavour. Protein concentrate is about 65-70% protein consisting of other non-protein components (Wang *et al.*, 2004; Kalman, 2014). Protein isolates and concentrates offer enormous opportunities in the development of a new class of formulated foods (Eltayeb *et al.*, 2011). Protein isolates and concentrates are considered as a suitable substitute in food processing to the legume seed flour because they have better functional properties, are free from indigestible carbohydrates and toxic elements, and have a low flavour profile (Adebowale & Lawal, 2003). Protein isolates and concentrates have high protein content hence, they have become more popular in the food industry. Their protein content ranges from 38 to 90% in some legumes (Rodriguez *et al* 2005). Some protein sources which have been reported for protein concentrates and isolates are Bambara groundnut flour, *Moringa oleifera* leaf powder, wheat flour, hemp, soy flour, brown rice flour, pea flour, cowpea, pigeon pea, mungbean, and hemp (Adebowale *et al.*, 2011; Sodamade & Adeboye, 2013; Diedericks *et al.*, 2019). Although legume proteins are good sources of essential amino acids, they are limiting in sulphur-containing amino acids. Complementing Bambara groundnut protein isolates (BGNPI) with *Moringa oleifera* leaf protein isolate MOLPI) may produce a food ingredient with a well-balanced amino acid profile.

Bambara groundnut (BGN) is rich in protein and essential amino acids. The value of BGN can be significantly enhanced through fractionation. BGN can be separated into starch, soluble fibre, insoluble fibre, and protein concentrate for use in value-added products (Adebowale *et al.*, 2002; Eltayeb *et al.*, 2011; Oyeyinka *et al.*, 2015; Maphosa, 2016; Gulu, 2018). BGN contains 15- 25% protein (Ajayi & Lale 2000; Baryeh 2001; Sirivongpaisal, 2008; Eltayeb *et al*., 2011; and Oyeyinka *et al.*, 2015). Methods reported for the extraction of protein isolate or concentrate from BGN flour include alkaline extraction/isoelectric precipitation, micellar precipitation, air classification, salt extraction-dialysis, water extraction, and ultrafiltration (Fan & Sosulski, 1974; Adebowale *et al.*, 2007; Boye *et al.*, 2010; Oujifard *et al.*, 2012; Kudre *et al.*, 2013; Stone *et al.*, 2015). Alkaline extraction/isoelectric precipitation is mostly preferred because it results in a good yield and in less time. Several authors have reported the protein content of Bambara protein isolate and concentrate to vary from 57 to 91% (Adebowale *et al.*, 2011; Eltayeb *et al.*, 2011; Kudre *et al.*, 2013; Arise *et al.*, 2015).

Moringa oleifera (MO) is a good source of protein, 9.38 – 16.7 g/100g was reported for the fresh leaf (Elmoneim *et al.*, 2007; Sohaimy *et al.*, 2015). The dried leaves of MO contain 30.3% crude protein and 19 amino acids (Moyo *et al*., 2011). Variation in protein content of MO leaves is attributed to environment, genetic background, and methods of cultivation (Sohaimy *et al.*, 2015). The crude protein of MO is close to that of sunflower seed, which is usually, used as protein concentrates or isolates. Methods used in the extraction of protein concentrate from MO include ethanol extraction, methanol extraction, alkaline extraction/precipitation, water extraction, and heat treatment (Sodamade and Adeboye, 2013; Ahmed, 2016). The protein content of *Moringa oleifera* leaf protein concentrate ranges from 38 to 39% as reported by Sodamade & Adeboye (2013) and Ahmed (2016).

Complementing BGNPI and MOLPI requires a proper understanding of the interaction between the two protein isolates in a solution and their phase behaviour (Giancone *et al.*, 2009). A phase is any homogeneous and physically distinct region that is separated from another region by a unique border (Brady *et al.*, 2017). It describes the complex interaction between physically distinct, separable portions of matter called phases that are in contact with each other. Some of the factors that may influence phase separation are biopolymer characteristics (reactive sites present, protein/polysaccharide-type, molecular weight, and charge density), biopolymer concentration and ratio, and solvent condition [pH and ionic strength] (BeMiller, 2011). The stability of biopolymer dispersion depends on the functional properties of individual biopolymer, nature, and strength of the protein-polysaccharide and polysaccharides-polysaccharides interaction in bulk solutions. The equilibrium situation exhibited by a binary mixture of protein/hydrocolloid in an aqueous solution may be miscibility, thermodynamics incompatibility segregative interaction, and complex coacervation (complexation or associative interactions). Segregative interaction occurs when the interaction is repulsive resulting in the separation of the polymers into two phases (Giancone *et al.*, 2009; Gupta *et al.*, 2012). Therefore, the objective of this chapter was to investigate the phase behaviour of BGNPI and MOLPI in solution and subsequently determine the optimum mix of the two protein isolates.

3.2 Materials and Methods

3.2.1 Sources of materials and equipment

The BGN seeds were purchased from Triotrade, Johannesburg, South Africa, and dried MO leaf powder was purchased from Moringa Africa, Johannesburg. Chemicals were purchased from Merck Pty Ltd, South Africa. The equipment was obtained from the Department of Food Science and Technology, Cape Peninsula University of Technology, Cape Town, South Africa.

The major equipment used in this study include; a cabinet dryer (Model 1069616), a manual Corona Laders Y CIA A.8 extruder, a stainless steel flour mill (Fritsch, serial-no 19.1020/0152 Germany), centrifuge (Avanti® J-E centrifuge JSE111330, Beckman coulter Inc,

USA), orbital shaker and magnetic stirrer. Kenwood food processor blender, Turbiscan, MA 2000 apparatus, a discovery Hybrid rheometer, Thailand), and Zetasizer, (NanoZS, Malvern instruments). Figure 3.1 outlines the analyses that were carried out in this chapter.

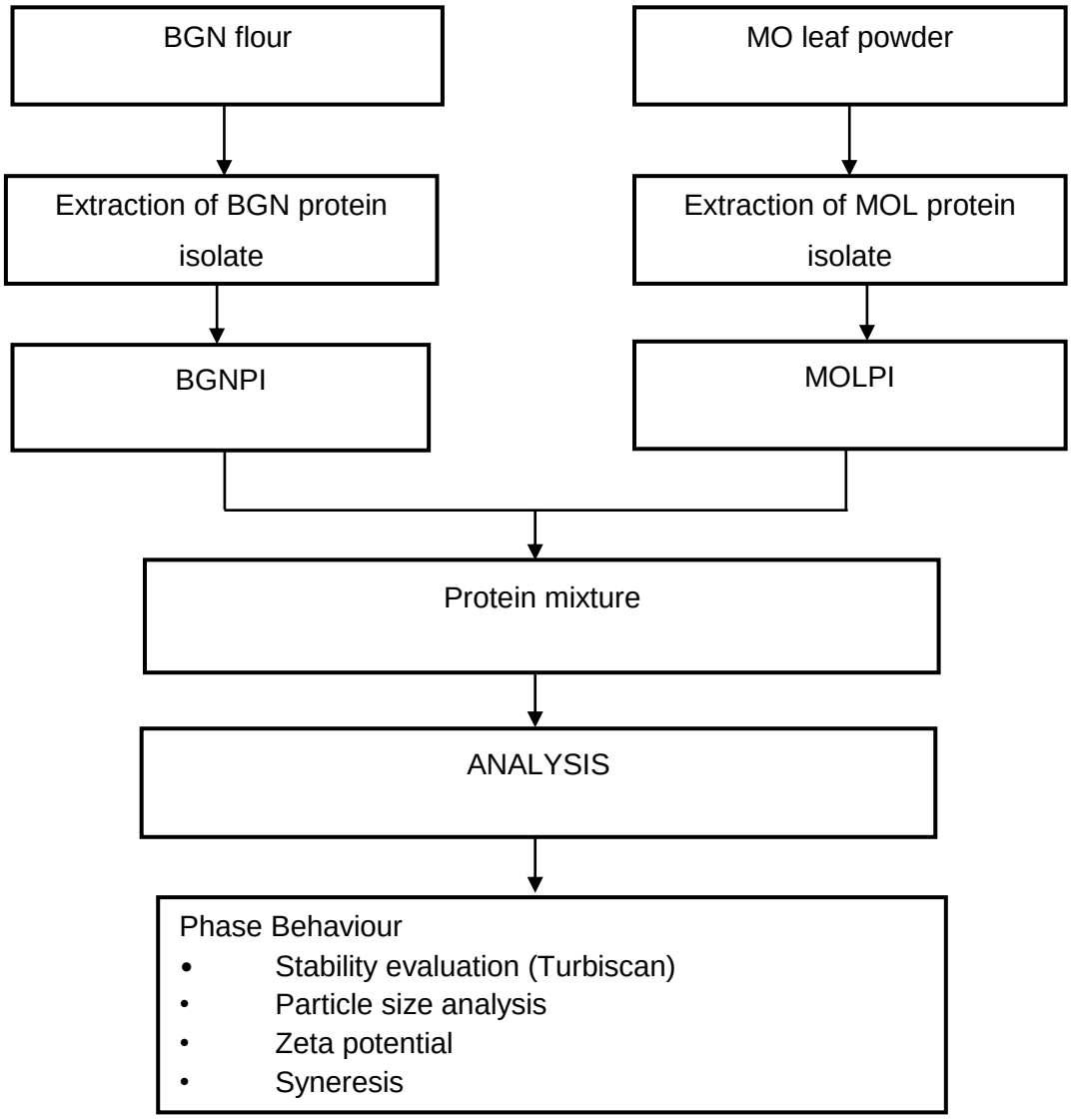


Figure 3.1 Chapter 3 analysis outline. BGN = Bambara groundnut; MO = Moringa olefera; MOL = *Moringa oleifera* leal; BGNPI = bambara groundnut protein isolate; MOLPI = *Moringa oleifera* leaf protein isolate

3.3 Preparation of Protein Isolates

3.3.1 Production of Bambara groundnut flour

The BGN seeds were sorted to remove defective ones and eliminate physical hazards such as stones. The seeds were dried (cabinet dryer, Model: 1069616) at 50^{o}C for 48 h. The dried seeds were crushed using a manual Corona Laders Y CIA A.8 extruder and milled to flour

using a stainless steel mill (Fritsch, serial-no 19.1020/0152 Germany) with a sieve size of 0.75 mm. The BGN flour was packed and stored in a zip-lock bag at refrigeration temperature (4-6^{o}C) until required.

3.3.2 Extraction of Bambara groundnut protein isolate from Bambara groundnut flour

The isoelectric precipitation method as described by Wang *et al.* (2004), Kudre *et al.* (2013), and Adebowale *et al*. (2011) was used for extraction with modification. Figure 3.2 gives a flow diagram of the BGN protein isolate extraction procedure. The BGN flour was defatted by soaking 600 g in 80% ethanol (1:2 w/v), for 2 h. Thereafter sieved with a muslin cloth and the residue was dried in the fume cupboard for 2 h. Defatted BGN flour (200 g) was dispersed in 2 L millipore water and blended for 5 min in a Kenwood food processor blender at speed 3. The pH was adjusted to 8.5 with 2 M NaOH. The mixture was stirred continuously for 30 min at 60^{o}C using a magnetic plate at 1500 rpm, followed by centrifugation at 14 000 x g for 30 min at 15^{o}C. The supernatant was collected, and the pH was adjusted to 4.5 with 2M HCl and refrigerated at 4^{o}C for 1 h. The precipitate formed was recovered by centrifugation at 14 000 x g for 30 min at 4^{o}C. The precipitates obtained were freeze-dried and kept in a zip lock bag at 4^{o}C until further analysis.

The procedure was repeated with some modification without the application of heat where the pH of the slurry obtained after blending was adjusted to 9 with 1 M NaOH. The mixture was stirred continuously for 30 min at room temperature using a magnetic stir plate at 1500 rpm, followed by centrifugation at 10 000 x g for 30 min at 5^{o}C. Two additional extractions were carried out with half of the initial volume of water as described previously. The supernatants were combined and the pH was adjusted to 5 and kept in the refrigerator for 1 h before centrifugation. The precipitate formed was recovered by centrifugation at 10 000 x g for 15 min at 5^{o}C. The procedure with no heat application was repeated, where solubilisation was carried out at pH 8.5 and precipitation at pH 4.5.

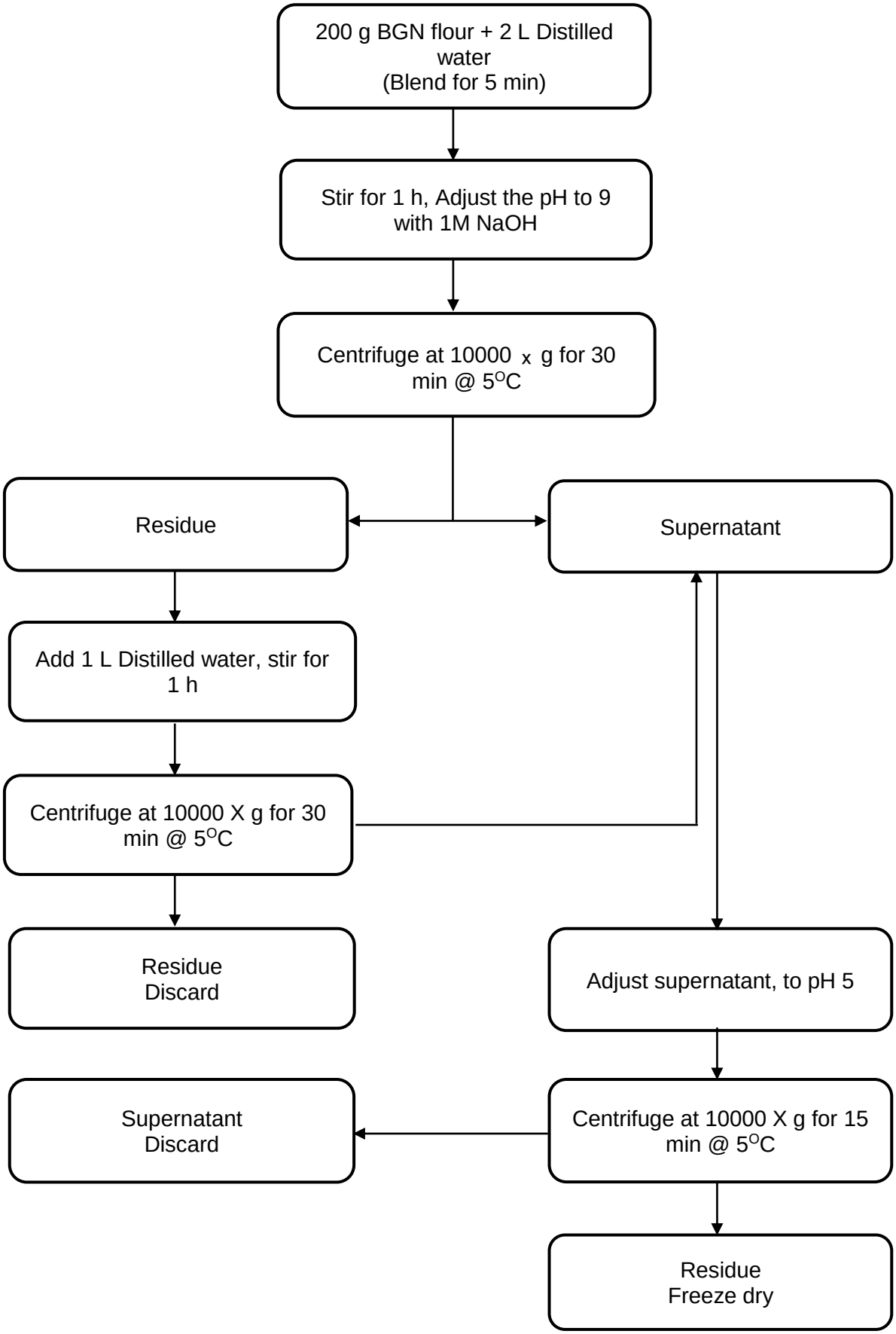


Figure 3.2 Flow diagram of BGN protein isolate extraction procedure.

3.3.3 Extraction of *Moringa* protein isolate from *Moringa oleifera* leaf

Multiple methods were employed to select the best method with the highest yield for the extraction of protein isolate from *Moringa oleifera* leaf powder (MOLP) as shown in Figure 3.3. The multiple methods employed were (1) metabisulphite (2%) in sodium citrate buffer at pH 8; (2) metabisulphite (2%) in sodium citrate buffer at pH 6; (3) sodium citrate buffer at pH 6; (4) solubility and precipitation at pH 7; (5) solubility and precipitation at pH 12; (6) sonication; (7) homogenization and (8) heat treatment.

The method described by van de Velde *et al.* (2011) was used with modification for the first three methods. A total of 50 g of MOLP was blended with 150 ml 2% metabisulphite ($Na_2S_2O_5$) in sodium citrate ($Na_3C_6H_5O_7$) buffer at pH 8 for method 1; 150 ml 2% $Na_2S_2O_5$ in $Na_3C_6H_5O_7$ buffer at pH 6 for method 2; and 150 ml $Na_3C_6H_5O_7$ buffer at pH 6 for method 3. The same procedure was applied to the slurry obtained in methods 1 to 3. The slurry was blended in a Kenwood food processor at speed no 3 for 5 min. The mixture was sieved with a muslin cloth; the filtrate was heated for 5 min at 60^{o}C, and centrifuged at 14 000 x g at 5^{o}C for 20 min. The supernatant was dialysed using a dialysis tube with a 10 KDa cut-off to remove sodium metabisulphite. The filtrate obtained was freeze-dried. The freeze-dried powder was packed and stored in a zip-locked bag at 4^{o}C until further analysis.

The method described by Ursu *et al.* (2014) was employed for solubility and precipitation at pH 7 and 12 (methods 4 and 5). The first stage of dissolving and blending MOLP applies to methods 6 and 7. A 200 g of dried MOL powder was blended with 1.5 L distilled water in a Kenwood food processor blender at speed 3 for 5 min. The slurry obtained was adjusted to pH 7 (method 4) and pH 12 (method 5) with 1 M NaOH, thereafter was centrifuged at 10 000 x g for 30 min at 5^{o}C. The supernatant was precipitated at pH 4 with 1 M HCl and centrifuged at 10 000 x g for 30 min at 5^{o}C. The resultant precipitate was freeze-dried. As stated above, the procedure for methods 4 and 5 was followed to obtain the slurry for methods 6 (sonication) and 7 (homogenisation). The slurry was sonicated for 1 h at 40^{o}C using an ultrasonic water bath, followed by centrifugation at 10 000 x g for 30 min at 5^{o}C. The resultant precipitate was freeze-dried. For method 7 (Homogenisation), the slurry was obtained as previously described and then subjected to homogenisation in a 2-stage mode homogenizer (GEA Niro Soavi Manual-TWINPANDA 400 NS2002H model). Homogenisation was performed at a pressure of 200 bar with an operating temperature of 20^{o}C. The feeder hopper was filled with water to clean the machine; afterwards, the MOLP extract was run. The resultant mixture was centrifuged at 10 000 x g for 30 min at 5^{o}C. The precipitate obtained was freeze-dried.

The heat treatment described by Fasuyi and Aletor (2005) and Aletor (2010) was used for method 8. Figure 3.4 gives a schematic diagram of the heat treatment method.

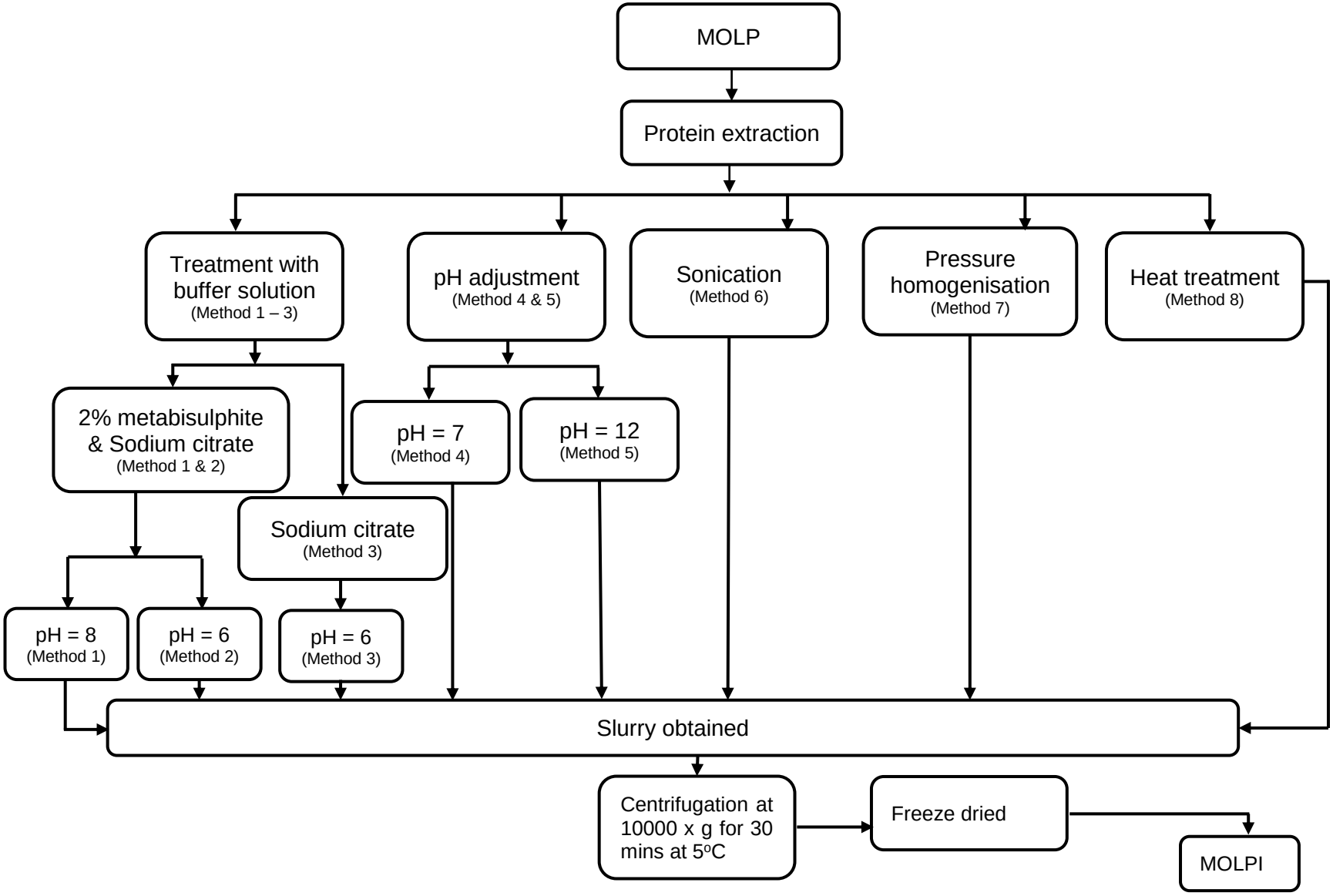


Figure 3.3 Multiple extraction methods for *Moringa oleifera* leaf protein isolate

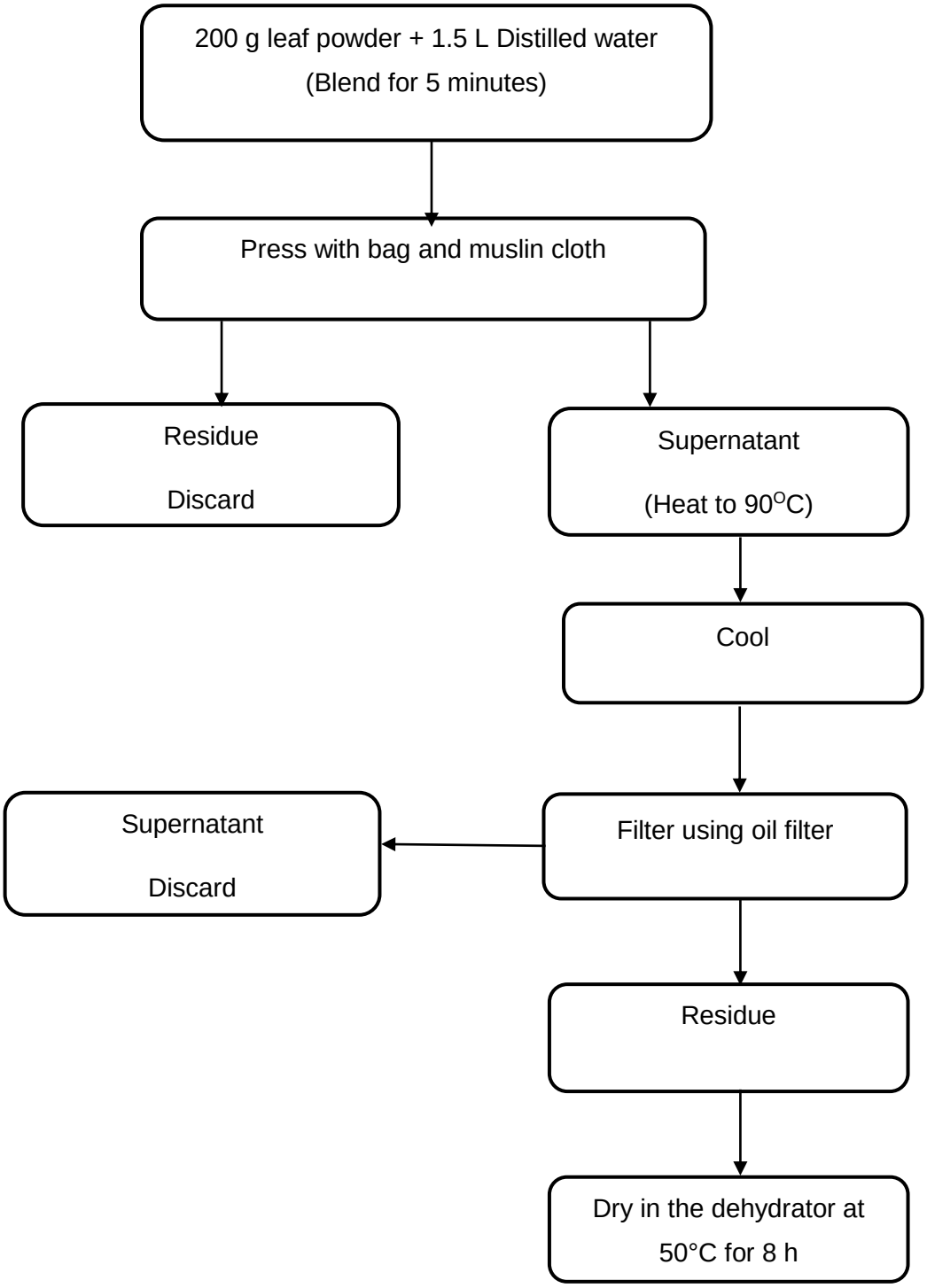


Figure 3.4 Extraction method for *Moringa oleifera* leaf protein isolate by heat treatment.

A 200 g of dried *Moringa oleifera* leaf powder (MOLP) was blended with 1.5 L distilled water in a Kenwood food processor blender at speed 3 for 5 min. The slurry obtained was pressed through a 10-micron and a 5-micron filter bag, respectively. The residue was washed again with 1.5 L distilled water, blended and the slurry was treated as was done previously to extract additional juice. The resultant juice was filtered through a muslin cloth. The juice was heated

to 90^{o}C to coagulate the proteins, the coagulum was filtered through oil filter bags, and the residue was dried in a digital food dehydrator with stainless steel trays at 50^{o}C for 8 h. After drying, the product (MOLPI) was milled into powder. The milled MOLPI was stored in a zip-lock bag, and stored at 4^{o}C until further analysis.

3.4 Phase Separation Analysis of Bambara groundnut and *Moringa oleifera* leaf protein isolate mixture

3.4.1 Experimental design for compatible mixtures of Bambara groundnut and *Moringa* protein isolates in aqueous solution

A 2^2 factorial experimental design augmented with centre points was used to determine the main effects of independent variables on the phase behaviour of the protein isolates in solution. The independent variables [Bambara groundnut protein isolate (X_1) and *Moringa* leaf protein isolate (X_2)], and their quantities are detailed in Table 3.1. The outline of the experimental design with the coded levels (-1 = low, 0 = middle, +1 = high) of independent variables are summarised in Table 3.2. Each design point was performed in duplicate with the centre in triplicates. Each design point was formulated by dissolving the protein isolates in 100 ml distilled water and homogenised at 10 000 rpm for 10 min using a D-lab homogeniser. The homogenised mixture was analysed for stability index, particle size, and zeta potential. Syneresis was investigated by visual observation. Consequently, the dependent variables of the experimental design were stability index, syneresis, and particle size.

3.4.2 Stability of Bambara groundnut protein and *Moringa* protein isolates in solution using Turbiscan

The stability of the protein mixtures was analysed using a Turbiscan vertical scan MA 2000. The method of Giancone *et al*. (2009) was used with modification. Suspension of Bambara groundnut protein isolate (BGNPI) and *Moringa oleifera* leaf protein isolate [MOLPI] (8 mL) from section 3.4.1 was transferred into a Turbiscan glass sample tube and closed tightly. The sample tube was inserted inside the cell. The detection head analyser scanned the entire height of the tube containing the sample over 2 h at 10 min intervals. The destabilisation was determined using a scanning analysing detection head for the identification of instability mechanisms such as coalescence or flocculation (due to particle size variation), as well as sedimentation and creaming (due to particle migration). The detection head passes light through the sample to the transmission detector, while the backscattering detector measures the light scattered backwards by the sample. Measurement was done in triplicate. The Turbiscan stability index (TSI) of the protein mixtures was obtained using equation 3.1 as described by Yao *et al* (2017).

$$TSI = \sqrt{\frac{\sum_{i=1}^{n}(X_i - X_{BS})^2}{n-1}} \qquad \text{Equation 3.1}$$

Where X_i is the average value of the scattered light intensity at each time of the instrument, X_{BS} is the average of X_i and n is the number of scanning. The lower the TSI value, the more stable the mixture.

Table 3.1 Independent variables for the 2^2 factorial augmented centre point design for BGNPI and MOLPI in a solution[1,2]

		Coded (x_i)		
Factors	Uncoded (X_i)	Low (-1)	Centre (0)	High (+1)
BGNPI	X_1	1	5	9
MOLPI	X_2	0.5	2.5	4.5

[1] BGNPI = Bambara groundnut protein isolate; MOLPI = *Moringa oleifera* leaf protein isolate

[2]Transformation of coded variable (x_i) to uncoded variable (Xi) levels could be obtained from $X_1 = 4x_1 + 5$; $X_2 = 2x_2 + 2.5$

Table 3.2 Design points for the 2^2 factorial augmented centre point experimental design of BGNPI and MOLPI mixture[1]

	Composition	
Run	BGNPI	MOLPI
1	-1	+1
2	+1	+1
3	+1	+1
4	+1	-1
5	0	0
6	-1	-1
7	+1	-1
8	0	0
9	0	0
10	-1	+1
11	-1	-1

[1]Coded levels (-1, 0, +1) correspond to the low, centre and high level, respectively. BGNPI = Bambara groundnut protein isolate (1, 5, 9% w/v); MOLPI = *Moringa oleifera* leaf protein isolate (0.5, 2.5, 4.5% w/v)

3.4.3 Particle size analysis and size distribution by dynamic light scattering

The particle size and size distribution (dispersity) were determined by dynamic light scattering using a dynamic light scattering (DLS) instrument (Zetasizer-nano series, Malvern Instruments Ltd, Malvern) according to the method described by Dai *et al.* (2017) with modification. The protein mixtures were diluted 10-fold with distilled water before measurement and particle size was measured in a polystyrene cuvette having non-opaque sides. The mean (Z average) of the sample represents the size of the particle and was equivalent to the mean volume distribution.

3.4.4 Particle dispersion stability by zeta potential measurement

The zeta potential to estimate the stability of the particle suspensions was determined by a Zetasizer (Malvern Instruments) according to the method of Dai *et al.* (2017) with some modifications. The protein mixtures were diluted 10-fold with distilled water before measurement. The diluted mixtures were injected into the cuvette for zeta potential measurement.

3.4.5 Syneresis

Syneresis was studied according to the method of Dai *et al.* (2017) with modification. Visual observation of the protein mixtures for phase separation was carried out after standing the mixtures for 4 h at 22^{o}C. Syneresis of the suspensions was calculated using equation 3.2.

$$\text{Syneresis } (\%) = {}^{V_p}\!/_{V_t} \times 100 \qquad \text{Equqtion 3.2}$$

Where Vp represents the volume of the precipitated layer (ml), and V_t, total volume (ml) of the protein mixture.

3.5 Numerical optimisation

The method described by Ngemakwe *et al.* (2015) was used for numerical optimisation. The data (stability index, syneresis, and particle size) obtained from the behaviour of BGNPI and MOLPI in solution as described in section 3.4.1 were fitted to a linear interaction model regression (equation 3.3) using Design-Expert version 11.

$$Y = \beta_0 + \sum_{k=1}^{2} \beta_i X_i + \sum_{k=1}^{2} \beta_{ij} X_i X_j + \varepsilon \qquad \text{Equation 3.3}$$

Where Y is the response variable (stability index, particle size, and syneresis), β_0 is a constant; β_i and β_j are the main effects, and β_{ij} are the interactive coefficients, X_i and X_j are the levels of BGNPI and MOLPI, respectively and ε is the random error. The quality of the fit of the linear

model equation was evaluated by the coefficient of determination (R^2), predicted R^2, adjusted R^2, adequate precision (AP), and lack of fit. The regression coefficients were used to examine the impact of each independent variable on the dependent variable.

Numerical optimisation was used to search for the optimum mixture of BGNPI and MOLPI. The optimisation objective was to minimise MOLPI and maximise the stability index. MOLPI has a bitter taste; hence, it is important to use the lowest possible concentration in food product so that its bitter taste does not overpower other ingredients.

3.6 Data Analysis

All experiments were conducted in triplicate and expressed as mean ± standard deviation. To determine the mean differences between treatments, the experimental data were subjected to Multivariate Analysis of Variance (MANOVA). Where differences existed, separation of means was carried out using Duncan's multiple range test (IBM SPSS, version 25).

3.7 Results and Discussion

3.7.1 Protein content and yield of Bambara groundnut and *Moringa oleifera* protein isolates

The protein content and yield of BGNPI from different extraction methods are shown in Table 3.3. Isoelectric precipitation was employed in the extraction of BGNPI with and without heat application. Protein content and yield varied significantly ($p < 0.05$) amongst the methods and BGNPI extracted with heat treatment gave the highest protein content (83.2%) with the lowest yield of 7.7 and 45.8% on flour and protein basis, respectively. Extraction with no heat treatment was solubilised at pH 8.5 and 9, thereafter precipitated at pH 4.5 and 5. The extraction at pH 8.5 and 4.5 resulted in higher protein content but lower yield compared to the extraction at pH 9 and 5. The extraction process and the final product are shown in Figure 3.5. The protein content (83.2%) obtained by heat extraction for BGNPI is comparable to 85.2% reported by Kudre *et al.* (2013) in which the isolate was extracted at pH 12 and 4.5 at 28-30^{o}C. However, BGNPI protein content of 70.5% and 61.1% obtained without heat treatment were both lower compared to 86.0% for BGNPI extracted from whole BGN flour without heat treatment as reported by Eltayeb *et al.* (2011). The variation in protein content among researchers may be attributed to BGN varieties, climatic conditions, type of soil, processing, and methods of determination. An extra fine milling step and lower sieve size (0.6 mm) were utilized in their study compared to the 0.75 mm sieve size, which was used in this present study. The higher protein may result from the flour to water extraction ratio of 1:15, and a high-speed stirrer, which could have enhanced better protein recovery compared to 1:10 used in our study. It could also be due to the absence of fat in the isolate obtained by the method of Eltayeb *et al* (2011) in which the fat was completely removed by n-hexane in a soxhlet

Table 3.3 Protein yield and content of Bambara Groundnut isolates extracted with heat or without heat treatment*

Extraction method	Protein content, %	Protein yield, % based on	
		Flour	Protein content
No heat treatment @ pH 8.5 and 4.5	70.5 ± 0.46[a]	10.3 ± 0.68[a]	61.5 ± 4.08[a]
Heat treatment	83.2 ± 0.70[b]	7.7 ± 0.76[b]	45.8 ± 4.56[b]
No heat treatment @ pH 9 & 5	61.1 ± 1.32[c]	16.0 ± 0.34[c]	95.6 ± 2.06[c]

*Data are mean ± standard deviation of triplicate determinations. Means within a column followed by different superscripts differ significantly ($p \leq 0.05$).

apparatus. Unfortunately, hexane is not applicable in this study, as the isolate will be applied in a food product. Hexane is top on the list of hazardous solvents, a health hazard due to high toxicity and contributes to environmental problems such as air pollution, fire, and explosion (Liu and Mamidipally, 2005; Virot *et al.*, 2008). Consequently, 80% ethanol was used to remove fat from BGN flour in this study.

BGN contains polyphenol as reported by several researchers in previous studies (Ademiluyi and Oboh, 2011; Mazahib *et al.*, 2013; Yao *et al.*, 2015; Gbenyi *et al.*, 2016; Nyau *et al.*, 2017; Mubaiwa *et al.*, 2019). Polyphenols are known to form a complex with protein (Gbenyi *et al.*, 2016), and are released during pre-processing such as soaking and also during cooking (Mazahib *et al.*, 2013). Therefore, the use of whole BGN flour, which was not pre-processed by soaking in this study, is an indication of the possibility of polyphenol in BGN flour binding to the extracted protein isolates. Some of the polyphenols were possibly released by heat. This also explained the brownish colour of the isolate as shown in Figure 3.5. Phenolic compounds are reported to be the major cause of the dark colouration of canola protein isolates and their removal resulted in protein isolates that are lighter in colour (Xu and Diosady, 2002). Pre-treatments such as hydration and cooking enhance the reduction of antinutritional compounds in legumes (Qayyum *et al.*, 2012).

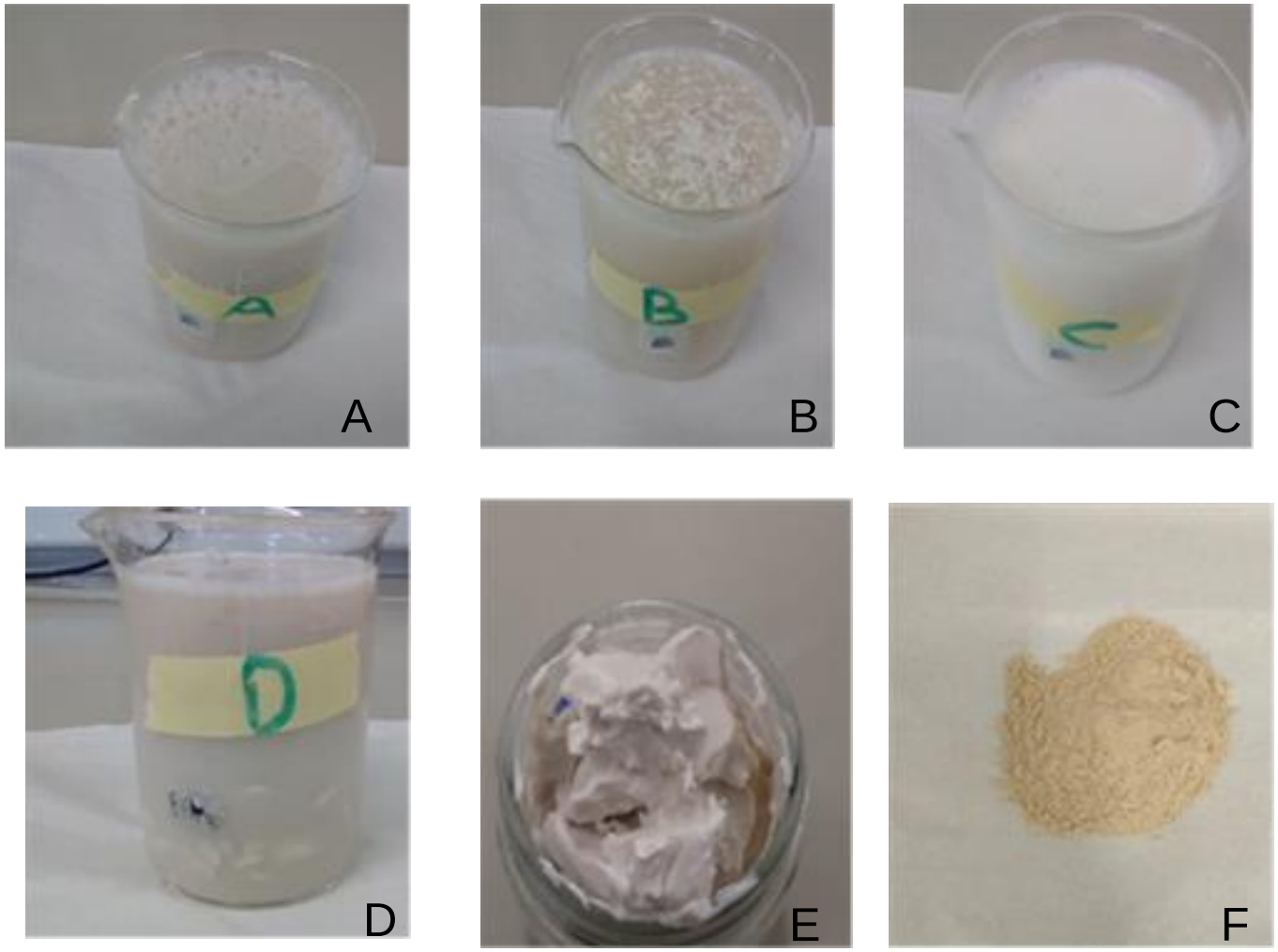


Figure 3.5 Extraction process of Bambara groundnut protein isolate (BGNPI). A and B: Solubilisation stages; C and D: Precipitation stages E: BGNPI before drying; F: BGNPI after drying;

Multiple methods (Table 3.4) were employed in the extraction of protein from *Moringa oleifera* leaves. The multiple methods aimed to arrive at a method that will give a substantial yield. There was a significant ($p < 0.05$) difference between the various methods as well as between the protein contents and the yield (Table 3.4). Heat treatment gave the highest yield of 20.9% and 65.7% on a flour and protein basis, respectively. Extraction by solubilisation and precipitation at pH 12 gave a yield of 4.3% and 13.5% on a flour and protein basis, respectively. The yield for the other 6 methods was very low, and there was no significant difference between them.

Extraction by solubilisation and precipitation at pH 7 and pH 12 resulted in the highest protein content of 55.0 and 57.3% and they did not differ significantly. Homogenization gave a protein content of 44.7% and it is significantly ($p < 0.05$) different from all the other methods. Treatment by sonication, heat application, 2% metabisulphite and sodium citrate buffer at pH 6, and 2% sodium citrate buffer at pH 6 resulted in protein content of 40.2, 39.4, 36.3, and 35.6%, respectively and did not differ significantly in protein content but the yield by heat treatment differed significantly. Treatment with 2% metabisulphite and sodium citrate at pH 8 gave the lowest protein content (30.27%), and it is significantly ($p < 0.05$) different from all the methods.

Table 3.4 Protein yield and content of *Moringa oleifera* leaf protein isolate extracted by different methods[1]

		Protein yield, % based on	
Extraction methods	Protein content, %	Flour	Protein content
2% $Na_2S_2O_5$ + $Na_3C_6H_5O_7$ @ pH 8	30.3 ± 0.05[a]	2.2 ± 0.32[a]	7.0 ± 1.01[a]
2% $Na_2S_2O_5$ & $Na_3C_6H_5O_7$ buffer @ pH 6	36.3 ± 1.10[b]	2.2 ± 0.20[a]	6.9 ± 0.63[a]
$Na_3C_6H_5O_7$ buffer @ pH 6	35.6 ± 5.00[b]	1.9 ± 0.06[a]	5.9 ± 0.18[a]
Solubilisation and precipitation @ pH 7	55.7 ± 1.54[d]	2.2 ± 0.12[a]	7.0 ± 0.38[a]
Solubilisation and precipitation @ pH 12	57.3 ± 1.64[d]	4.3 ± 0.02[b]	13.5 ± 0.07[b]
Sonication	40.2 ± 0.39[b]	1.4 ± 0.05[a]	4.4 ± 0.16[a]
Homogenistion	44.7 ± 4.00[c]	1.2 ± 0.00[a]	3.7 ± 0.15[a]
Heat treatment	39.4 ± 0.54[b]	20.9 ± 1.80[c]	65.7 ± 0.67[c]

[1]Data are mean ± standard deviation of triplicate determinations. Means within a column followed by different superscripts differ significantly ($p > 0.05$)

MOLPI obtained with sodium citrate buffer gave an unacceptable dark colour. However, the addition of sodium metabisulphite to sodium citrate buffer resulted in a better colour. Sodium metabisulphite was used as an antibrowning agent to improve the colour of the isolate, the use of sodium metabisulphite as an antibrowning agent is supported by literature (Ibrahim *et al.*, 2004; Sgroppo *et al.*, 2010). Extraction of MOLPI by heat treatment was selected because it gave the highest yield, the extraction process, and the final product is shown in Figure 3.6. The protein content of 39.4% obtained in this present study by heat application method is in good comparison with 41.7% reported by (Aletor, 2010) and 47.0% (Fasuyi and Aletor, 2005) for protein concentrates from cassava leaves. Chemical and structural changes like denaturation and aggregation occur in protein due to heat treatment (Bu *et al.*, 2009). Phenolic compounds are released from protein during heat treatment (Nyau *et al.*, 2017).

3.7.2 Selection of optimal extraction method for Bambara groundnut and *Moringa oleifera* protein isolates

The optimum extraction method for Bambara groundnut protein isolate (BGNPI) was selected based on solubility. Protein solubility of BGNPI extracted with heat application and without heat was investigated within pH 2–11. The lowest solubility of both isolates was at pH 4–5. The protein was soluble in the low acidic and the alkaline pH range. Kudre *et al* (2013) and Adebowale *et al* (2011) reported similar results for BGNPI. Protein molecules are charged at pH values far from the isoelectric point (pI), thus causing repulsion among proteins, which enhances increased solubility. Whereas net charges of protein give zero when the pH is close to isoelectric point (pI) (Kaur and Singh, 2007; Kudre *et al.*, 2013).

Isolates extracted without heat showed the highest solubility of 114.97% at pH 2 while the heat extracted isolates had the highest solubility of 97.07% at pH 6 (Figure 3.7). The isolate extracted without heat application is preferred because of better solubility. Some denaturation seems to have taken place during the heating process which was evident in the solubility result of BGNPI in which heat was applied (even though it has higher protein content and lower solubility compared to the products obtained without application of heat)

The effect of the extraction methods on the solubility of BGN protein isolates is shown in Figure 3.7. BGN protein isolate extraction methods used had a significant difference ($p = 0.001$) on the percentage protein solubility. As seen on Figure 3.7, the protein isolation method without heat application yielded BGN protein isolate with a higher percentage solubility compared to the BGN protein isolates extracted with heat application. However, the protein contents of isolates extracted with and without heat application were not significantly different (Figure 3.8). Therefore, the extraction method with no heat application is chosen as the best for this project.

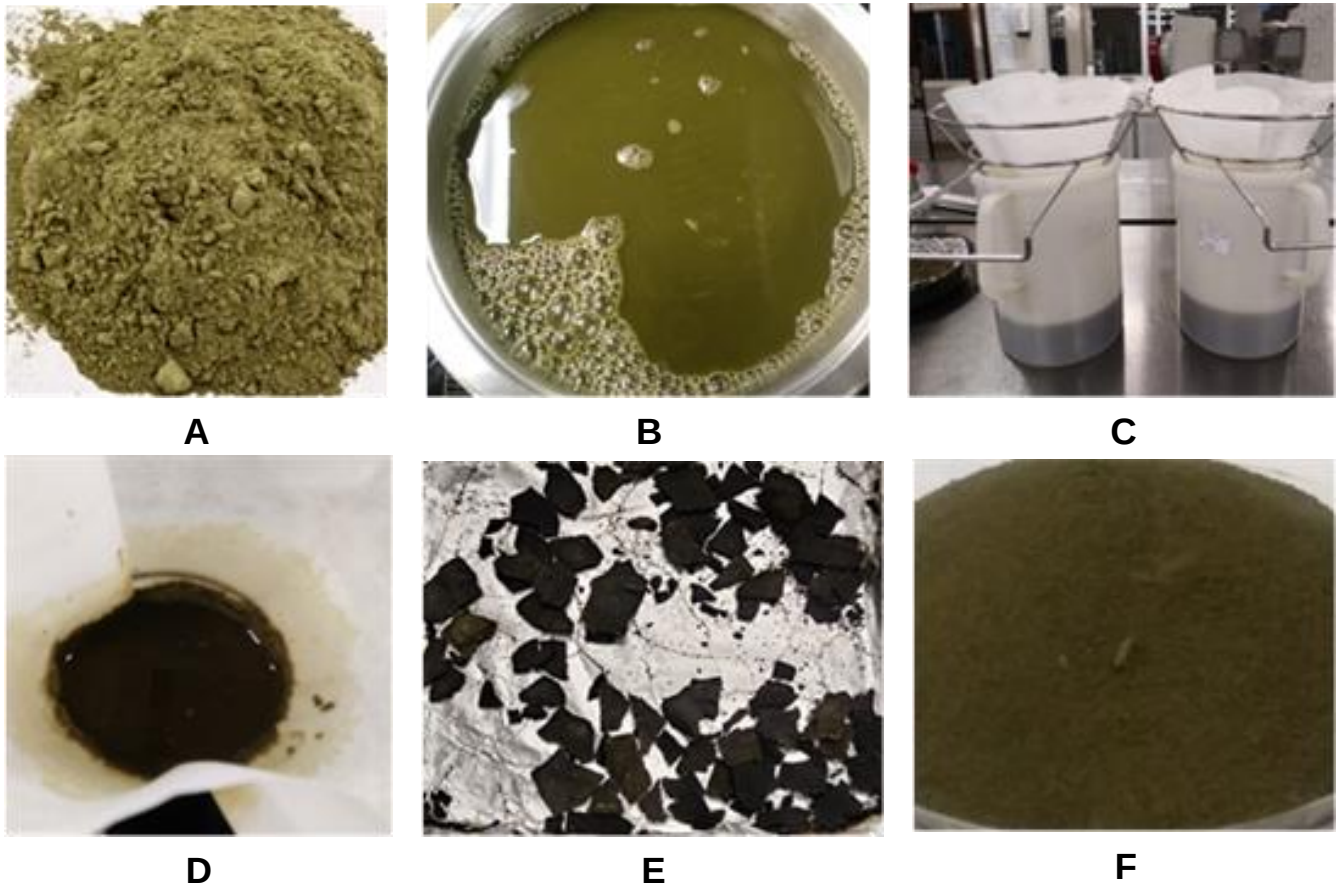
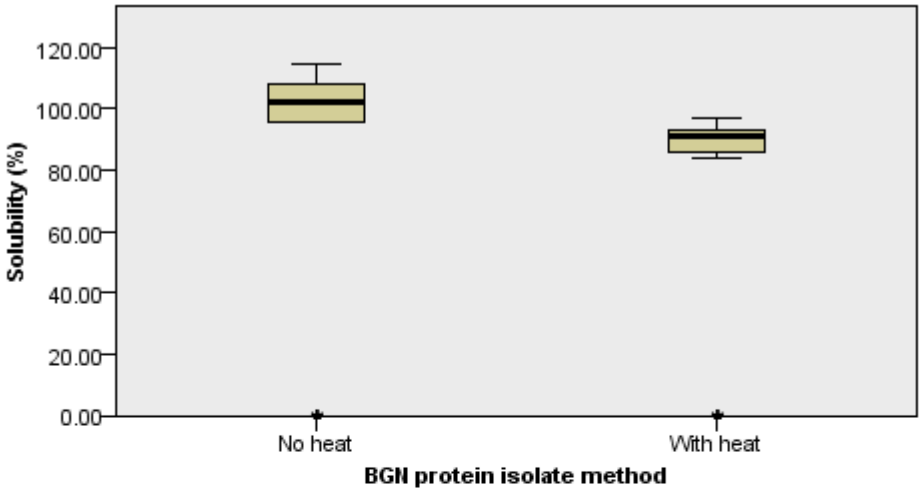


Figure 3.6 Extraction process of *Moringa oleifera* leaf protein isolate (MOLPI) by heat treatment. A: MOLPI leaf powder: B: Juice extraction stage, C and D: Filtration, E: Drying; and F: MOLPI after drying

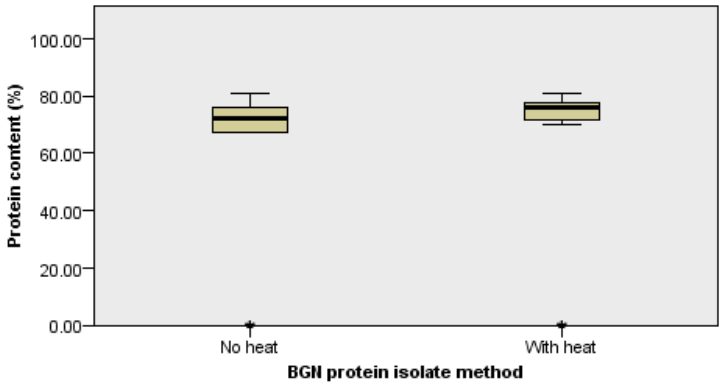
The optimal extraction method for *Moringa oleifera* leaf protein isolate (MOLPI) was selected in consideration of the method with the highest yield of protein isolate. Extraction by heat treatment having 65.7% yield was chosen



Total N	40
Test Statistic	11.711
Degrees of Freedom	1
Asymptotic Sig. (2-sided test)	.001

1. The test statistic is adjusted for ties.
2. Multiple comparisons are not performed because there are less than three test fields.

Figure 3.7 Comparison of the extraction methods of Bambara groundnut isolates concerning solubility.



Total N	40
Test Statistic	1.240
Degrees of Freedom	1
Asymptotic Sig. (2-sided test)	.265

1. The test statistic is adjusted for ties.
2. Multiple comparisons are not performed because the overall test does not show significant differences across samples.

Figure 3.8 Comparison of the extraction methods of Bambara groundnut isolates with protein content

3.7.3 Model adequacy

The effects of independent variables on the phase behaviour of BGNPI and MOLPI protein in solution are outlined in Table 3.5. The linear model regression coefficients are detailed in Table 3.6. The linear model was significant ($p = 0.0320$) for stability index and syneresis ($p = 0.0352$) in explaining the variation between the independent and dependent variables but not significant ($p = 0.1186$) for particle size. The adequacy of precision (estimation of signal to noise ratio) for stability index, syneresis, and particle size were 5.39, 4.11, and 4.43, respectively. A ratio of 4 is desired. These values being greater than 4 indicated that the models were adequate. The significant lack of fit for stability index, syneresis, and particle size were 0.6270, 0.4108, and 0.0339, respectively indicated that lack of fit was not significant for stability index and syneresis, and hence the model was adequate for both. But the lack of fit was significant ($p < 0.05$) for particle size, which further indicates that the model was not adequate for particle size. The adjusted coefficient of determination (R^2) for stability index, syneresis, and particle size were 0.6946, 0.4052, and 0.5451 indicating variation in the fit for the models. The adjusted coefficients of determination (R^2adj) for syneresis exhibited better goodness of fit compared to other parameters (Table 3.6). In general, the models were

Table 3.5 Effects of BGNPI and MOLPI on stability index, particle size, and syneresis[1]

	Isolate		Response variables		
Run	BGNPI	MOLPI	Stability index	Syneresis (%)	Particle size (d.nm)
A	-1	-1	0.0348	88.9 ± 0.0	964.2 ± 165.5
B	-1	+1	0.0094	38.7 ± 33.2	1804.7 ± 648.7
C	+1	-1	0.0413	63.0 ± 33.6	578.8 ± 263.4
D	0	0	0.0238	55.8 ± 17.9	414.1 ± 176.0
E	+1	+1	0.0048	16.2 ± 5.7	757.2 ± 310.6

[1]Coded levels (-1, 0, +1) correspond to the low, centre and high level, respectively. BGNPI = Bambara groundnut protein isolate (1, 5, 9% w/v); MOLPI = *Moringa oleifera* leaf protein isolate (0.5, 2.5, 4.5% w/v)

Table 3.6 Regression coefficients of the linear model for the phase behaviour of BGNPI and MOLPI in a solution[1,2].

	Response variable		
Coefficients	Stability Index	Syneresis (%)	Particle size (d.nm)
Linear			
β_0	-3.1273		729.9889
β_1	0.0246	-11.3958	-37.8203
β_2	-0.3600		230.8177
Interaction			
β_{12}	-0.0206		-20.6927
R^2	0.6946	0.4052	0.5451
***p*-value**	0.0320	0.0352	0.1186
Adjusted R^2	0.5637	0.3391	0.3501
AP	5.392	4.106	4.432
Lack of fit	0.6270	0.4108	0.0339

[1] β_0 = constant, β_1 = effect of BGNPI, β_2 = effect of MOLPI, β_{12} R^2 = coefficient of determination, AP, Adequate Precision

[2] BGNPI, Bambara groundnut protein isolate; MOLPI, *Moringa oleifera* protein isolate

adequate in explaining the effect of mixture components (BGNPI and MOLPI) on the responses (stability index, syneresis, and particle size), and could be used to navigate the design space.

3.7.4 Main and interaction effects of BGNPI and MOLPI in solution on stability index, syneresis, and particle size of Bambara groundnut and *Moringa oleifera* protein mixture

The linear model regression coefficients for each response variable for the phase behaviour of BGNPI and MOLPI in solution (Table 3.6). BGNPI had a significant (p = 0.032) effect on the stability index (0.0206) but did not have a significant effect on syneresis and particle size (Table 3.6). As the level of BGNPI increased from 1 to 9 g (Figure 3.9), there was a decrease in stability index which indicates better stability. The lower the stability index, the more stable the solution. This implies that increasing the concentration of BGNPI in the protein mixture lowers the stability index resulting in better stability of the mixture. BGNPI can stabilise the protein mixture due to its natural stabilising components, and it could achieve better stability at a higher concentration. Many researchers have reported the ability of Bambara groundnut flour [BGNF] (Adeyi *et al.*, 2014, 2019; Fasinu *et al.*, 2015) to stabilise an emulsion, and others reported the ability of Bambara groundnut soluble fibre to stabilise beverage emulsion (Diedericks and Jideani, 2015; Maphosa *et al.*, 2016). The researchers also reported that better stability is achieved at increasing concentration. However, BGNPI could not significantly affect syneresis and particle size. It could be that the concentration of BGNPI used was not sufficient to obtain the required viscosity that would prevent syneresis (Donato & Guyomarc'h, 2009).

MOLPI did not have a significant effect on stability index and particle size and did not affect syneresis (Table 3.6). As the level of MOLPI increased from 0.5 to 1.9 g (Figure 3.9), there was a decrease in stability index, which is an indication of stability, but as the level of MOLPI increased further from 2 to 4.5 g, the effect of MOLPI on stability flattened. MOLPI not having a significant effect on the stability index means that its stabilising ability cannot singly stabilise the protein mixture.

There was an interaction between BGNPI and MOLPI in the solution. The combined effect of BGNPI and MOLPI significantly decreased ($p < 0.05$) the stability index, with no effect on syneresis, and significantly increased ($p < 0.05$) the particle size (Figure 3.9). Low particle size is an indication of better stability; increased particle size thus increased instability of the system. This means that the synergistic combination of BGNPI and MOLPI lowered the stability index and resulted in better stability of the protein mixture but their combined interaction was not sufficient to prevent syneresis. The combined effect of BGNPI and MOLPI resulted in increased particle size. This explains why the major destabilisation phenomenon observed in the protein mixture was flocculation (particle size variation). Better stability of the protein mixture can be achieved by using hydrocolloids; researchers have reported the use of

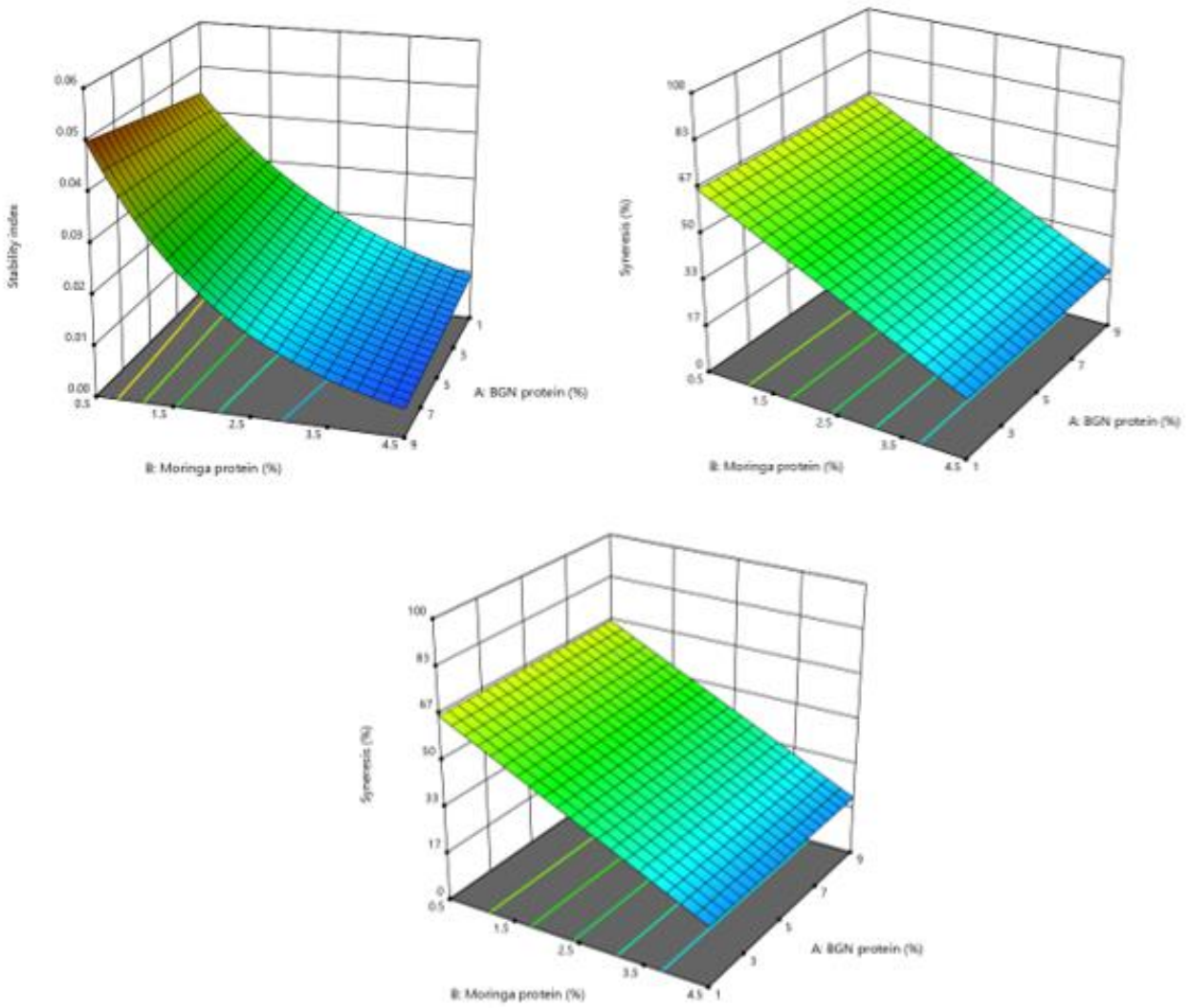


Figure 3.9 Effect of BGN protein and *Moringa oleifera* leaf protein on a: Stability index; b: syneresis; and c: particle size of MOLP and BGN protein mixture. BGN: Bambara groundnut, MOLP: *Moringa oleifera* leaf protein.

hydrocolloids in stabilising emulsion (BeMiller, 2011; Phimolsiripol *et al.*, 2011), and can be effective for the protein mixture as well.

3.7.5 Stability of Bambara groundnut and *Moringa oleifera* leaf protein mixture.

Figure 3.10 are the Turbiscan profiles of Bambara groundnut protein isolate (BGNPI) and *Moringa oleifera* leaf protein isolate (MOLPI) different mixtures. The profiles indicate the stability and instability curves as backscattering (BS) flux percentage (%). The BS % of protein mixtures are presented on the y-axis and the tube length on the x-axis. Figure 3.10a consists of low concentration of both protein isolates [(1%) BGNPI, (0.5%) MOLPI]. Figure 3.10b consist of 1% BGNPI (low concentration) and 4.5% MOLPI (high concentration); Figure 3.10c: 9% BGNPI (high concentration) and 0.5% MOLPI (low concentration); Figure 3.10d: 5% BGNPI and 2.5% MOLPI (both at centre point concentration); and Figure 3.10e consists of 9% BGNPI and 4.5% MOLPI (both at high concentration). There was a variation in the profiles, which indicates that the particles were not evenly dispersed in the mixture. For a stable product, the backscattering graph will show little or no variation in thickness, all the profiles will overlay as one curve (Formulaction smart scientific analysis, 2009). Variation of the thickness of the backscattering graph indicated particle size variation or particle migration. Flocculation indicates particle size variation that is demonstrated by a horizontal thick line. While sedimentation and creaming indicate particle migration, which was demonstrated by the vertical thick line at the bottom and top of the mixture, respectively.

All the protein mixture showed a peak from 0-7 mm, an indication of particles settling at the bottom of the tube due to sedimentation. Scans of protein mixture in Figure 3.10a, representing the lowest concentration of dispersed protein isolates showed sedimentation, flocculation, and creaming (Figure 3.10). Scans of protein mixture in Figure 3.10b with 1% BGNPI (low concentration) and 4.5% MOLPI (high concentration) followed the same pattern but the variation in particle size reduced compared to protein mixture in Figure 3.10a. Instability observed with the protein mixtures in Figure 3.10c, consisting of 9% BGNPI (high concentration) and 0.5% MOLPI and 3.10d with 4: 5% BGNPI and 2.5% MOLPI (both at centre point concentration) was majorly sedimentation and flocculation, and also with less particle size variation compared to what was observed in Figures 3.10a and 3.10b. Scans of protein mixture in 3.10e represent protein mixture with 9% BGNPI and 4.5% MOLPI (both at high concentration) showed some level of stability, the mixture was stable between 30-70 mm as observed on the x-axis of Figure 3.10. The instability mechanism that occurred in this study were sedimentation, flocculation, and creaming. Prevalent destabilisation phenomenon in BGN flour and BGN fibre was likewise observed to be flocculation and coalescence ((Adeyi *et al.*, 2014; Diedericks, 2014; Maphosa, 2016). It could be assumed that the protein mixtures represented in Figure 3.10e are the most stable mixture, which was also confirmed by the stability index (0.0048) and syneresis (16.2%) (Table 3.5). Both stability index and syneresis.

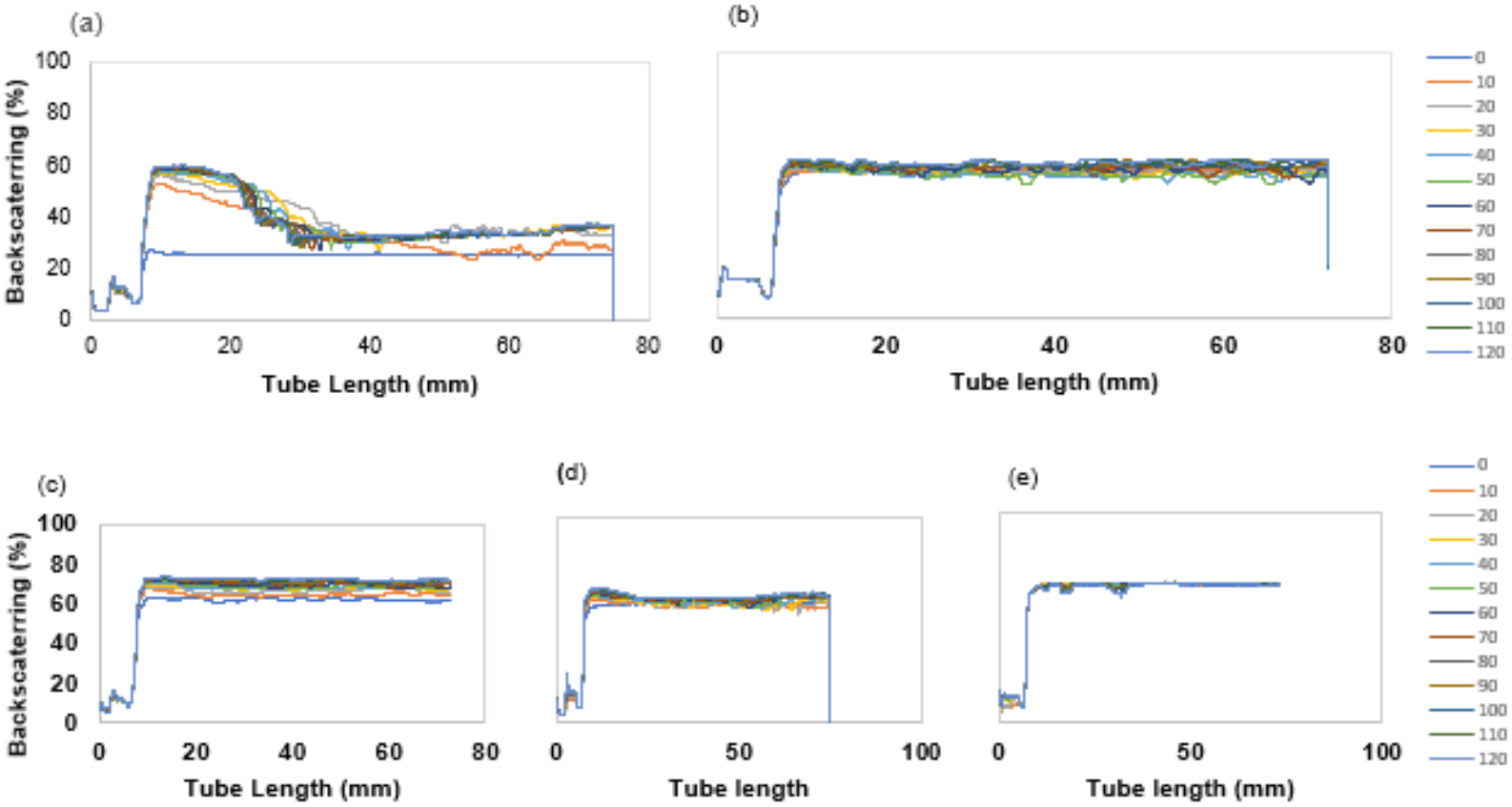


Figure 3.10 Turbiscan backscattering multiple stability scan profile of BGN and MO protein mixture: (a) protein mixture 1 (1% BGNPI, 0.5%MOLPI), (b) protein mixture 2 (1% BGNPI, 4.5% MOLPI), (c) protein mixture 3 (9% BGNPI, 0.5% MOLPI), (d) protein mixture 4 (5% BGNPI, 2.5% MOLPI), and (e) protein mixture 5 (9% BGNPI, 4.5% MOLPI)

of protein mixture represented by Figure 3.10e have the lowest value, which is an indication of better stability (Formulaction smart scientific analysis, 2009)

The destabilisation of the protein mixtures observed in Figures 3.10a and 3.10b could be due to the interaction of the dispersed phase with the continuous phase. Creaming occurs when the density of the dispersed phase is lower than the continuous phase. It is therefore not surprising that creaming occurred in protein mixtures in Figures 3.10a and 3.10b because they have a composition with lower quantities of the dispersed phase (Table 3.5). Creaming was coupled with flocculation and there was a phase separation. Also, two proteins in solution interact with each other and with other molecules such as lipids and carbohydrates. The interactions can result in thermodynamic incompatibility, which may be associative or segregative. Associative phase separation occurs through electrostatic repulsion when oppositely charged polymers are involved (Giancone *et al.*, 2009). When the polymers are carrying the same charge, segregative phase separation occurs if the polymer concentration is higher than the co-solubility limit. This behaviour may be generalised for every binary macromolecules mixed system. Less instability is observed with protein mixture (Figure 3.10c) when BGNPI is at the highest concentration (9%) compared to 3.10b when the concentration of BGNPI is 1%. Adeyi *et al.*(2014) reported that emulsion stability is enhanced with the increasing concentration of BGN flour. Improved stability when both isolates were mixed at centre point concentration (BGNNPI-5%, MOLPI-2.5%) and better stability when both were at the highest concentration (BGNPI-9%, MOLPI-4.5%). It is observed that BGNPI at high concentration improved the stability but MOLPI does not. This is in agreement with the linear model regression in section 3.7.4. The impact of MOLPI on stability index is static at a higher concentration from 2% to 4 .5%, indicating that MOLPI has no effect on the stability of the protein mixture at high concentration, and this might be the reason for the little instability observed with protein mixture Figure 3.10e. MOLPI demonstrated more impact around 2% concentration.

Stability observed with protein mixture in Figure 3.10e could be due to the higher composition of BGNPI and MOLPI in the mixture (Table 3.1). Instabilities observed may also be as a result of the pH of the protein mixtures which was around 5, the emulsifying capacity of BGNPI and MOLPI are improved at alkaline pH than the acidic (Adebowale et al., 2011).

3.7.6 Particle size and zeta potential of the protein isolate

The particle size of protein mixtures diluted 10-fold was determined as Z-average value by volume using the Dynamic Light scattering (DLS) technique. Effects of the protein isolates on particle size distribution are represented in Figures 3.11. Figure 3.11a represents protein mixture with the lowest concentration of dispersed phase (1% BGNPI, 0.5% MOLPI), followed by Figure 3.11b which has the least concentration of BGNPI and the highest concentration of

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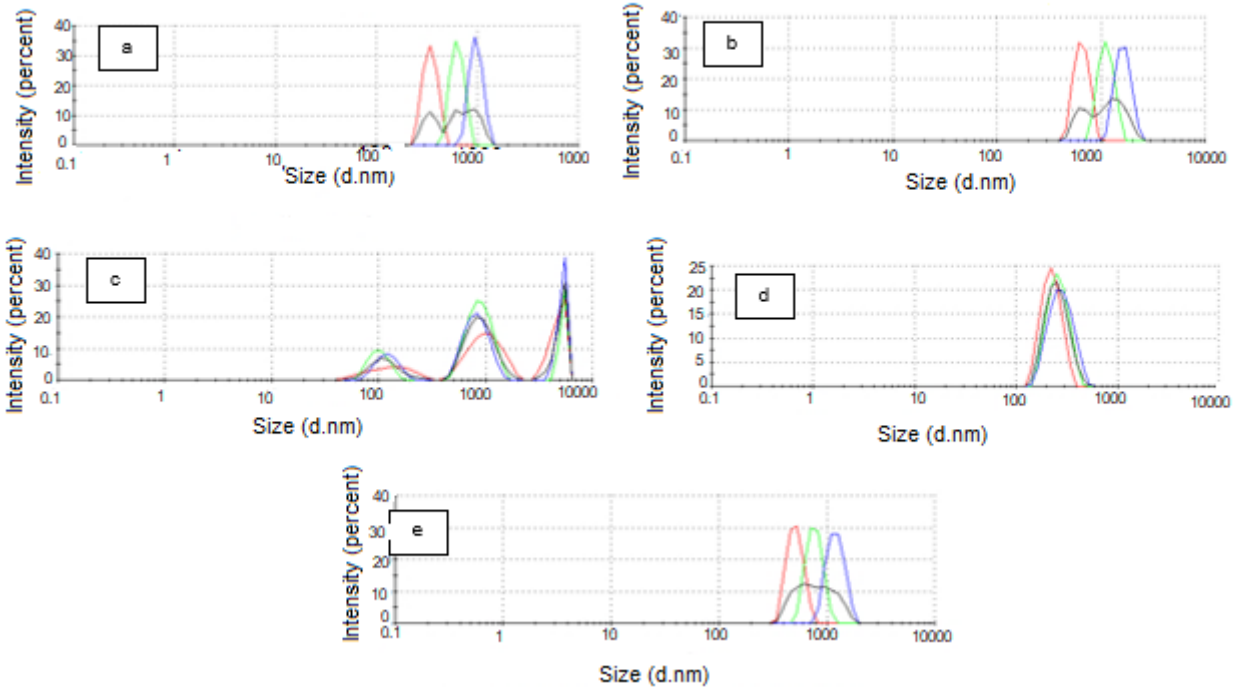

Figure 3.11 Particle size distribution of BGN and MOL mixture: (a) protein mixture 1 (1% BGNPI, 0.5%MOLPI), (b) protein mixture 2 (1% BGNPI, 4.5% MOLPI), (c) protein mixture 3 (9% BGNPI, 0.5% MOLPI), (d) protein mixture 4 (5% BGNPI, 2.5% MOLPI), and (e) protein mixture 5 (9% BGNPI, 4.5% MOLPI).

MOLPI (1% BGNPI, 4.5% MOLPI), Figure 3.11c consists of the highest concentration of BGNPI and lowest concentration of MOLPI (9% BGNPI, 0.5% MOLPI), Figure 3.11d has the centre point concentration of both BGNPI and MOLPI (5% BGNPI, 2.5% MOLPI) and Figure 3.11e consists of the highest concentration of both protein isolates (9% BGNPI, 4.5% MOLPI).

Protein mixture in Figure 3.11a, b, and e showed bimodal size distribution with the particle size 964, 1805, and 757 d.nm respectively, the protein mixture represented by Figure 3.11c showed multimodal with particle size 579 d nm, and the protein mixture in Figure 3.11d showed monomodal size distribution with particle size 414 d.nm (Figure 3.11, 3.12). None of the graphs showed a perfect bell shape and tended to have a shoulder which could be an indication of a second population indicating coalescence. This could mean that all the protein mixtures were poly-dispersed, which means the particles have many sizes. There is no information in the literature about the stability of BGNPI and MOLPI in a solution, however, the effect of Bambara groundnut flour and Bambara groundnut fibre in emulsion stability have been reported (Adeyi *et al.*, 2014; Maphosa *et al.*, 2016), and destabilisation due to coalescence was also observed in their studies. It could be assumed that protein mixture represented by Figure 3.12b is the least stable because larger particle size in the system resulted in a greater coalescence having a higher impact and magnitude during the collision (Adeyi, 2014). Protein mixture in Figure 3.11d and Figure 3.12d with the centre point concentration of both BGNPI and MOLPI (Figure 3.11, 3.12) could be deduced to have the most stable system as it has a narrow size distribution and less particle size. Smaller sizes are more stable than larger sizes and narrow distributions are generally more stable than broader distribution. Smaller particle size of protein mixture represented by Figures 3.12d, c and e could be as a result of increasing concentration of BGNPI and MOLPI in them, this is in line with the previous report that the average size of emulsion is decreased and the more stable emulsion is obtained with increasing concentration of dispersed phase (Cheong *et al.*, 2016). Nevertheless, there is variation in observation from Turbiscan stability profiles and particle size by dynamic light scattering (DLS), Turbiscan profiles indicated protein mixture represented by Figure 3.10e as the most stable (Figure 3.10), while particle size by DLS showed that protein mixture Figure 3.12d is the most stable (Figure 3.12). Badeggi *et al.* (2020) reported size variation between DLS and electron microscopy (TEM), sampling volume used for analysis could be the reason for such variation.

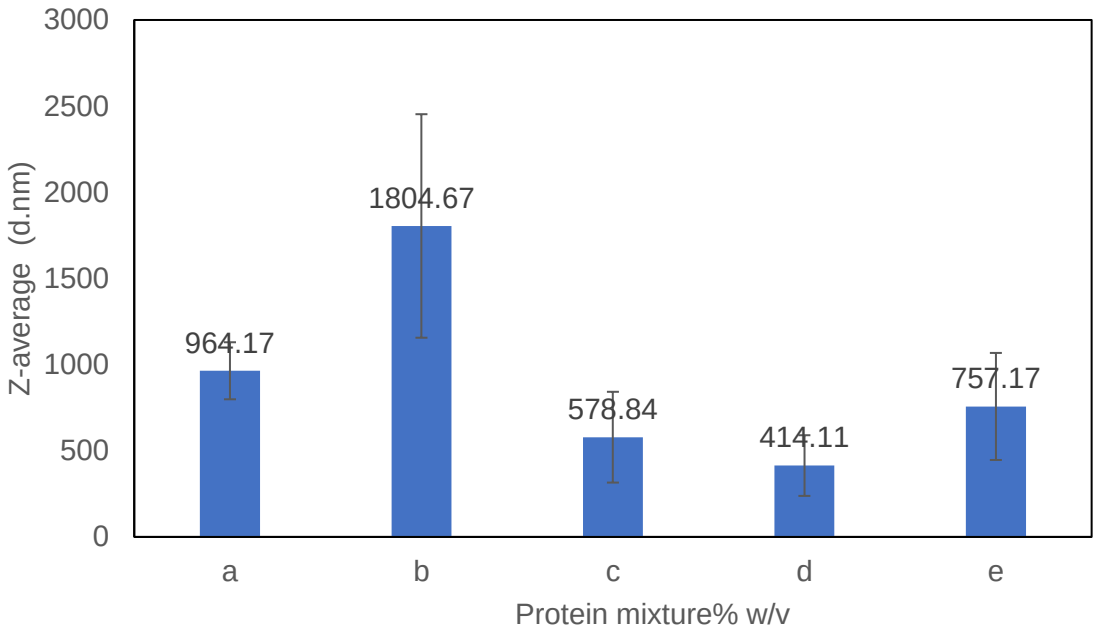

Figure 3.12 Particle size distribution of BGN and MOL protein mixture: (a) 1% BGNPI, 0.5% MOLPI), (b) protein mixture 2 (1% BGNPI, 4.5% MOLPI), (c) protein mixture 3 (9% BGNPI, 0.5% MOLPI), (d) protein mixture 4 (5% BGNPI, 2.5% MOLPI), and (e) protein mixture 5 (9% BGNPI, 4.5% MOLPI).

Zeta potential can be used as an indicator of stability, the zeta potential of the protein mixtures ranged from -0.37 to 9.15 mV. Low zeta potential promotes cell attachment and proliferation but high zeta potential (positive or negative) will confer stability because the particles will resist aggregation due to higher repulsive forces. The low values imply that aggregates are formed in all the protein mixture, and all have some level of instability. Zeta potential is essential in the characterisation of the stability of colloidal dispersion as it measures electrical repulsive forces between particles. A high zeta potential of ±30 mV is considered stable (Cheong *et al.*, 2016; Dai *et al.*, 2017). It is an indication of high repulsive force which prevents coalescence and results in better stability. For particle suspensions in water, zeta potential is the electrical potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle (Doostmohammadi *et al.*, 2011). Instability of the protein mixtures resulting from particle aggregation observed with Turbiscan stability analysis and DLS is also confirmed with the zeta potential.

3.7.7 Syneresis

All compositions of BGN and MO protein mixtures were homogeneous after mixing. Phase separation was observed with a cloudy layer and lighter brownish upper layers after standing for 4 h. Syneresis can be described as serum release or water release from gel matrix (Phimolsiripol *et al.*, 2011; Dönmez *et al.*, 2017) and for this study, it is the release of water from the protein mixtures. Syneresis is visual evidence for the instability of mixtures and a lower syneresis is an indication of greater stability (Dai *et al.*, 2017).

The appearance of BGN and MO protein mixtures observed visually after mixing and after standing for two hours is shown in Figure 3.13. Figure 3.13a represents protein mixture with the lowest concentration of dispersed phase (1% BGNPI, 0.5% MOLPI). Figure 3.13b has the least concentration of BGNPI (1%) and the highest concentration of MOLPI (4.5%I). Figure 3.13c consists of the highest concentration of BGNPI and lowest concentration of MOLPI (9% BGNPI, 0.5% MOLPI). Figure 3.13d has the centre point concentration of both BGNPI and MOLPI (5% BGNPI, 2.5% MOLPI) and Figure 3.13e consists of the highest concentration of both protein isolates (9% BGNPI, 4.5% MOLPI).

Protein mixtures represented by Figures 3.14a, b, c, d, and e showed syneresis 88.9, 21.3, 28.6, 55.8, and 21.7%, respectively (Figure 3.14). Greater syneresis (88.9%) was observed with protein mixture represented by Figure 3.14a when both isolates were at low concentrations. Lower syneresis was observed with protein mixture represented by Figure 3.14b (21.3%) and Figure 3.14e (21.7%) respectively. Protein mixture represented by Figure 3.14b consists of a lower concentration of BGNPI (1%) and the highest concentration of MOLPI (4.5%) and protein mixture represented by Figure 3.14e contains the highest concentration of both BGNPI (9%) and MOLPI (MOPI). The high concentration of isolates must have increased viscosity of the protein mixture leading to reduced syneresis in both protein mixtures represented by Figure 3.14b and 3.14e. Although the mixtures were initially homogeneous, protein rearrangement over the standing period might have resulted in syneresis (Donato and Guyomarc'h, 2009). Higher viscosity decreases syneresis, higher viscosity can be attained by the use of hydrocolloids. The use of hydrocolloids to reduce syneresis has been reported, this could be due to the lower water holding capacity of the isolates, however, hydrocolloid was not used in this study. The efficiency of hydrocolloid in decreasing syneresis was reported by several studies (Phimolsiripol *et al.*, 2011; BeMiller, 2011). The protein isolate mixture represented by Figures 3.14b and 3.14e (21.28 and 21.67% respectively) has the lowest syneresis, hence they are both considered as the most stable. The instability observed visually was supported by the Turbiscan profiles (Figure 3.10) and stability index (Table 3.6), where the protein isolate mixture represented by Figure 3.14e showed the least instability.

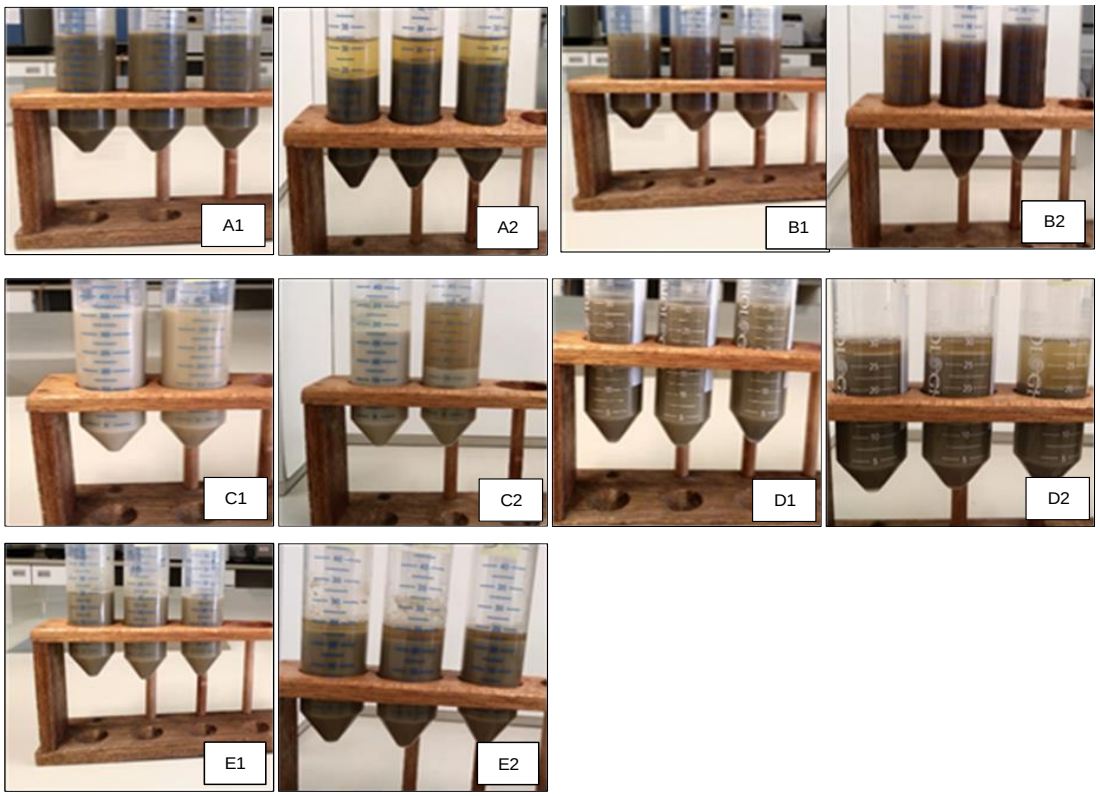

Figure 3.13 Appearance of the BGN and MOLP protein mixture observed visually after mixing (A1, B1, C1, D1, E1) and after standing (A2, B2, C2, D2, E2) for 4 hours at 22ºC

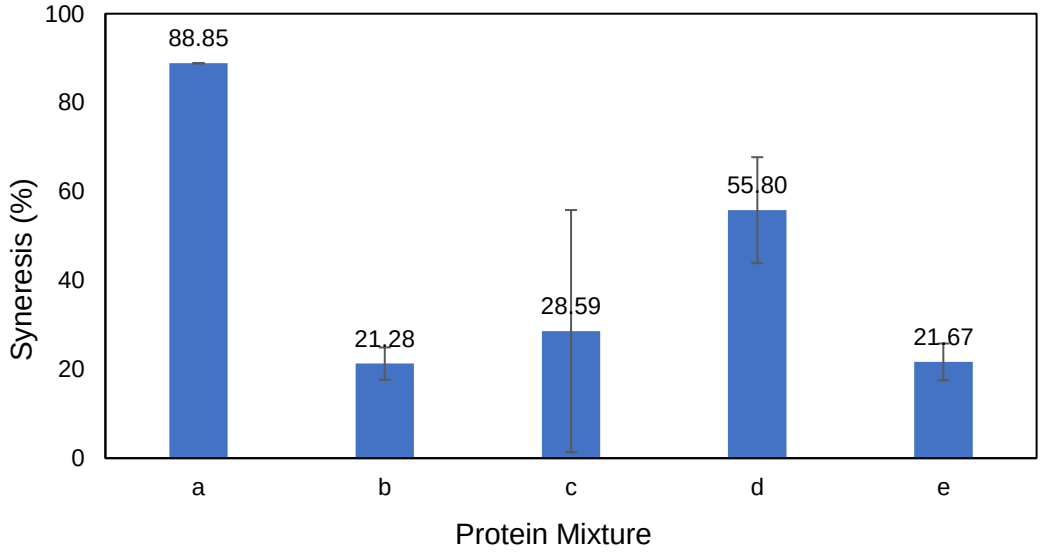

Figure 3.14 Syneresis of BGN and MOL protein mixture: (a) protein mixture 1 (1% BGNPI, 0.5%MOLPI), (b) protein mixture 2 (1% BGNPI, 4.5% MOLPI), (c) protein mixture 3 (9% BGNPI, 0.5% MOLPI), (d) protein mixture 4 (5% BGNPI, 2.5% MOLPI), and (e) protein mixture 5 (9% BGNPI, 4.5% MOLPI).

3.7.8 Optimisation of the protein isolates.

The optimization goal was to minimise *Moringa oleifera* protein isolate and stability index as shown in Table 3.8. The quantity of protein isolate for preparing the optimal protein isolate mixture was 9% BGN protein isolate and 2% MO leaf protein isolate in 100 ml distilled water with the desirability of 0.624.

3.8 Conclusion

Bambara groundnut protein isolate (BGNPI) and *Moringa oleifera* leaf protein isolate (MOLPI) were successfully produced from Bambara groundnut flour and *Moringa oleifera* leaf powder. BGNPI extracted by Isoelectric precipitation at pH 9 and pH 5 and MOLPI extracted by heat treatment are the most preferred and effective combinations that worked. Segregative phase separation was observed in all the protein mixtures. Sedimentation, creaming, and flocculation (particle size variation) were observed in all the protein mixtures. However, the protein mixture was stable to crea ming and sedimentation at a higher concentration of BGNPI and MOLPI. The major destabilisation phenomenon was flocculation. Greater instability of the protein mixture was at lower concentration of the isolates. The highest concentration of both BGNPI and MOLPI was the most stable protein mixture, which was further confirmed by stability index and syneresis (%). The concentration of BGNPI and MOLPI for the optimum mix was 9% BGNPI and 2% MOLPI. The objectives to produce BGNPI and MOLPI were actualised.

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CHAPTER FOUR
FUNCTIONAL AND NUTRITIONAL PROPERTIES OF BAMBARA GROUNDNUT AND *MORINGA OLEIFERA* LEAF PROTEIN COMPLEX

ABSTRACT

Bambara groundnut and *Moringa oleifera* leaf protein complex (BAMOLP) was produced using Bambara groundnut protein isolates (BGNPI) and *Moringa oleifera* leaf protein isolate (MOLPI). The protein isolates and complex (BGNPI, MOLPI, and BAMOLP) were subjected to physical tests (particle morphology, colour, and water activity), proximate analysis, amino acid analysis, functional tests (water absorption capacity, oil absorption capacity, foaming capacity, foaming stability, emulsifying capacity, emulsifying stability, swelling capacity, and water activity) and rheological test. The protein isolates and complex are non-newtonian and pseudoplastic. The oil absorption capacity (OAC) of BGNPI, MOLPI, and BAMOLP was 2.26, 0.89, and 0.95 g/g respectively while the water absorption capacity (WAC) for BGNPI, MOLPI, and BAMOLP were 1.31, 1.5, and 1.22 g/g, respectively. The WAC and swelling capacity of the BGNPI, MOLPI, and BAMOLP increased with temperature. The foaming capacity was pH dependent. BAMOLP exhibited a higher emulsifying capacity and stability than BGNPI and MOLPI. The crude protein of BGN flour and *Moringa oleifera* leaf powder were 16.75 and 31.78% respectively. The protein, fat, ash, carbohydrate, and moisture content of the protein isolates and complex ranged from 39.42 to 63.51%, 2.19 to 11.52%, 1.60 to 7.09%, 24.07 to 51.29%, and 2.61 to 9.57% respectively and significantly ($p < 0.05$) differed from one another. The results show that BAMOLP exhibits a better amino acid profile, rheological and chemical properties than its precursors BGNPI and MOLPI. Conclusively, complementing BGNPI and MOLPI was successful as BAMOLP showed an improvement over its precursors.

4.1 Introduction

Bambara groundnut (BGN) is rich in protein and essential amino acids. The value of BGN can be significantly enhanced through fractionation into starch, soluble fibre, insoluble fibre, and protein concentrate for use in value-added products (Adebowale *et al.*, 2002; Eltayeb *et al.*, 2011; Oyeyinka *et al.*, 2015; Maphosa, 2016; Gulu, 2018). BGN contains 15.48-25% protein (Ajayi & Lale 2000; Baryeh, 2001; Sirivongpaisal, 2008; Eltayeb *et al*., 2011; Oyeyinka *et al.*, 2015) depending on the varieties and environmental factors. BGN is richer in essential amino acids such as leucine, isoleucine, methionine, lysine, valine, phenylalanine, and threonine than groundnut (Adegbola & Bamishaiye, 2011). However, BGN has a low content of sulphur-containing amino acids (methionine and cysteine) and tryptophan. Therefore, complementing

BGN with a leaf protein that is rich in methionine can be useful in overcoming this limitation and provide a well-balanced amino acid profile. *Moringa oleifera* (MO) is an example of a leaf protein that is rich in methionine.

Protein in MO fresh leaf was reported to be 16.7% (Elmoneim *et al*., 2007) and 9.38 g/100 g (Sohaimy *et al.*, 2015). Moyo *et al*. (2011) reported that dried leaves of MO contain 30.3% crude protein and 19 amino acids. Variation in protein content of MO leaves is attributed to environment, genetic background, and methods of cultivation (Sohaimy *et al.*, 2015). The crude protein of *Moringa oleifera* leaf powder is close to that of sunflower seed, which is usually, used as protein concentrates. MO leaves are a rich source of essential amino acids such as methionine, cystine, tryptophan, and lysine (Perumal & Klaus 2003; Sohaimy *et al.*, 2015). Oliveira *et al*. (1999) reported that the content of methionine and cysteine in MO seed is 43.6 g/kg protein. This value is very high and it is close to that of human milk, chicken egg, and cow milk. Consumption of BGN and MO can thus be increased by processing them into protein isolates that can be used in value-added products.

Proteins are significant in food processing and product development due to their functional, physicochemical, and nutritional properties that impact consumer acceptability. Protein isolates are sources of beneficial and active compounds, especially amino acids; hence they are used as functional ingredients to increase the nutritional quality of food products (Wani *et al.*, 2015). Protein isolate offers enormous opportunity in the development of a new class of formulated foods (Eltayeb *et al.*, 2011). These isolates are considered as a suitable substitute in food processing to the legume seed flour because they have superior functional properties, low flavour profile, and relative freedom from toxic factors and indigestible carbohydrates (Adebowale & Lawal, 2003). The best use of the legume protein isolates depends on the effectiveness of their functional properties, which are influenced by intrinsic factors such as composition and conformation of proteins, environmental factors, as well as methods and conditions of isolation.

Essential functional properties of protein ingredients include oil absorption, swelling capacity, water absorption, foam capacity, and foam stability, while the nutritional properties are the amino acid balance of the protein. Studies on the characterisation of BGN protein isolates have been reported with BGN protein isolates showing high protein content and a good balance of essential amino acids (Adebowale *et al.*, 2011; Eltayeb *et al.*, 2011; Kudre *et al.*, 2013a; Arise *et al.*, 2015; Kaptso *et al.*, 2015; Adeleke *et al.*, 2018; Diedericks *et al.*, 2020b, a). Minimum protein solubility was reported at pH 4-5 (Kudre *et al.*, 2013). The amino acid profile of *Moringa oleifera* leaf (MOL) was reported by Oiveira (1999), Ritcher (2003), and Busani *et al.* (2011). Protein isolate of MOL is nutritionally adequate for use as a food ingredient (Sodamade & Adeboye, 2013). Oluduro (2012) reported that MOL contains a substantial amount of nutrients. The application of MOL as a food ingredient in value-added products and

animal feeds has been reported, protein complex between BGN and MOL protein isolate was produced in chapter 3 of this thesis, however, nothing is known about its functionality as a food ingredient. Therefore, the objective of this chapter was to investigate the nutritional and functional properties of protein isolate extracted from Bambara groundnut (BGN) flour and *Moringa oleifera* leaf powder (MOLP) and their complex to establish the suitability of Bambara groundnut-*Moringa oleifera* leaf protein complex in ready-to-use-therapeutic foods (RUTF).

4.2 Materials and Methods

4.2.1 Sources of material and equipment

Bambara groundnut was purchased from Triotrade Johannesburg, South Africa and *Moringa oleifera* leaf powder was purchased from *Moringa* Africa, Johannesburg. Chemicals were purchased from Merck Pty Ltd, South Africa. The equipment was obtained from the Department of Food Science and Technology, Cape Peninsula University of Technology, Cape Town, South Africa. The major equipment used in this study includes stainless steel flour mill (Fritsch, serial-no 19.1020/0152 Germany), centrifuge (Avanti® J-E centrifuge JSE111330, Beckman coulter Inc, USA), orbital shaker, and magnetic stirrer. Truspec Nitrogen Analyser (LECO-Laboratory Equipment) USA, a discovery Hybrid rheometer, a polytron homogenizer (IKA T18, Ultra-Turrax, Bechthai, Bangkok, Thailand). Figure 4.1 outlines the analyses that were carried out in this chapter.

4.2.2 Production of Bambara groundnut and *Moringa oleifera* leaf protein complex

Bambara groundnut and *Moringa oleifera* leaf protein complex [BAMOLP] was produced by combining 9% BGNPI and 2% MOLPI. The isolates were obtained as detailed in section 3.8 of Chapter 3 and the combination for BAMOLP was established in Chapter 3 as the optimum mix for optimum stability of BGNPI and MOLPI in an aqueous solution. Both isolates were weighed, thereafter mixed in a blender until well combined. The complex was packed into a zip-locked bag; the bag was placed in an airtight container and thereafter stored at 4^{o}C in a fridge until further analysis.

4.2.3 Physical characterisation of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

The determination of morphology, water activity, and colour of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* leaf protein isolate (MOLPI), and Bambara groundnut-*Moringa oleifera* leaf protein complex (BAMOLP) are discussed in this section.

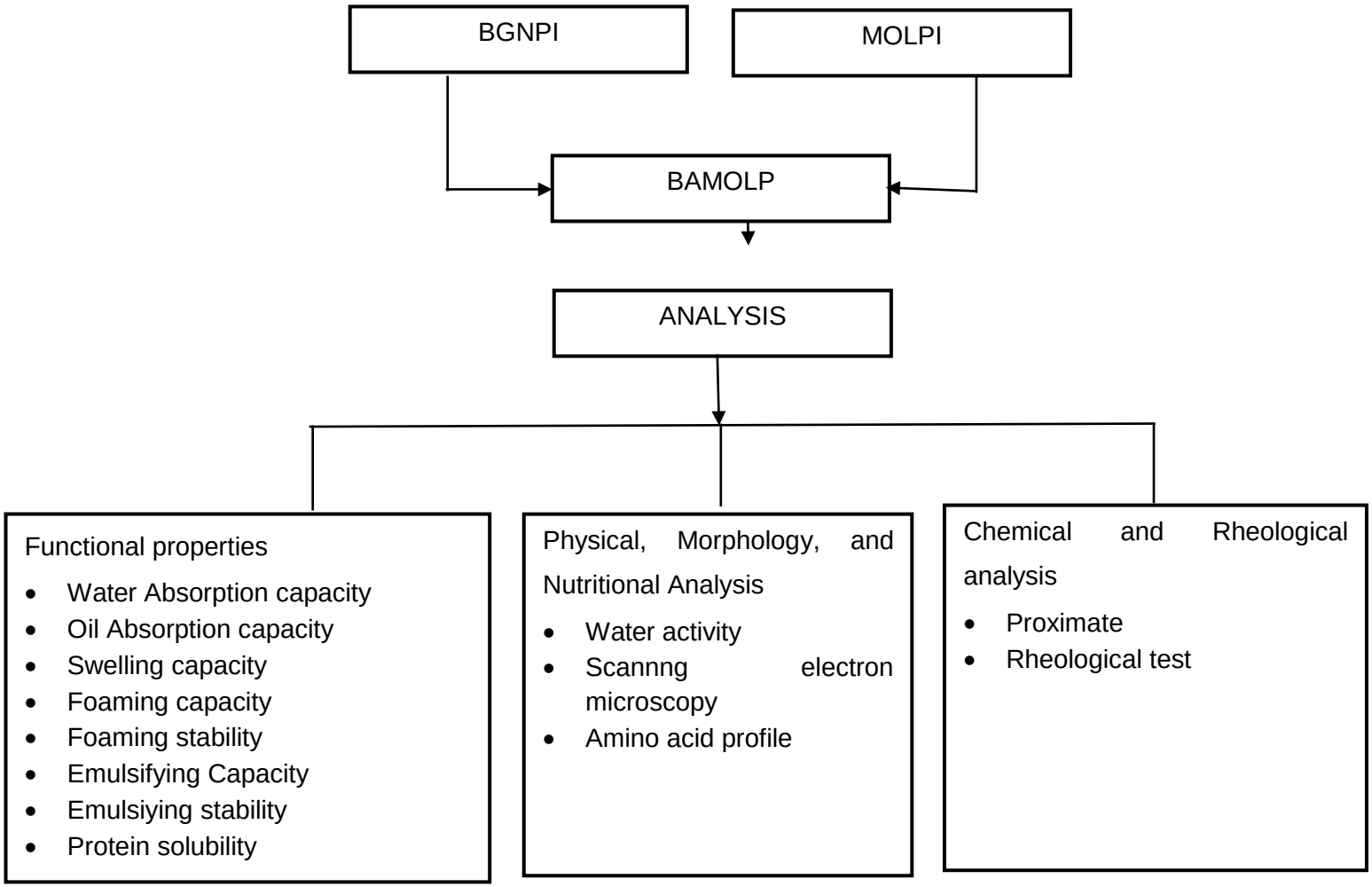

Figure 4.1 Chapter 4 outline. BGNPI: Bambara groundnut protein isolate, MOLPI: *Moringa oleifera* protein isolate, BAMOLP: Bambara groundnut, and *Moringa oleifera* protein complex

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4.2.4 Particle morphology determination of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex.

The morphology of the BGNPI, MOLPI, and BAMOLP was examined using a scanning electron microscope (SEM). Samples were separately placed on SEM stubs using double-sided tape. They were then sputter-coated with Gold/Palladium for 60 s at a sputter setpoint of 20 mA and pressure vacuum of 2 x 10^4 Torr. Scanning electron microscopy was conducted using the ThermoFischer Apreo FESEM. Images were acquired using the ETD detector, with the beam current set to 0.10 nA and a voltage of 2.00 kV. A working distance of 10.4 mm was maintained.

4.2.5 Colour determination of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

The colour of the protein isolates and complex was measured using a Spectrophotometer (Model CM-5 $45^o/0^o$ standard, Konica Minolta Sensing, Osaka, Japan), set at standard observer 10^o and D65. The instrument was calibrated using a black tile (L* = 5.49, a* = 7.08, b* = 4.66) and a white tile (L* = 93.41, a* = -1.18, b* = 0.75), followed by zero calibration. The L* coordinate is lightness, 100 represents white and closer to 0 represents black, a* (chromaticity coordinate +a* = red and –a* = green), b* (chromaticity coordinate +b* = yellow and –b* = blue), C* = Chroma, h = hue angle (0^o = +a*, 90^o = +b*, 180^o = -a* and 270^o = -b*).The isolates were milled to fine powder using a mortar and pestle, and a blender. The samples were placed in a sample-dish (30 mm diameter) and reflectance was measured for L*a*b* and L*C*h* colour space. Measurements were taken in triplicates for each sample (one reading = average of three readings per rotated position). The total colour difference (ΔE) was estimated using equation 4.1.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{Equation 4.1.}$$

4.2.6 Water activity determination of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

The water activity (a_w) of BGNPI, MOLPI, and BAMOLP was measured using the Novasina Ms 1 Set a_w meter, which uses a cell protection filter. The measurement cell was calibrated with salt humidity standards of 53, 75, and 90%. BGNPI, MOLPI, and BAMOLP were transferred individually into the sample container, and placed inside the Novasina analyser and the cell measuring protection filter was immediately closed. The water activity reading was observed for stability and the values were recorded (Novasina General Catalogue, 2012). The test was carried out in triplicate

4.3 Evaluation of functional properties of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

The functional properties of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* leaf protein isolate (MOLPI), and their complex were investigated in this section. Functional properties examined were water absorption capacity, oil absorption capacity, swelling capacity, foam capacity and stability, emulsifying capacity and stability, and protein solubility.

4.3.1 Water and oil absorption capacity of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

The method reported by Adebowale & Lawal (2004) was used for the determination of water and oil absorption capacity. Samples of BGNPI, MOLPI, and BAMOLP (1 g) each were mixed with 10 mL distilled water (or vegetable oil) in separate pre-weighed 50 mL centrifuge tubes. The sample was vortexed for 1 min, kept for 30 min at room temperature, thereafter separated by centrifuging at 4000 x g for 30 min. The supernatant was decanted and surplus water (or oil) in the upper phase was drained for 15 min. To determine the amount of water or oil retained per gram of the sample, the tube containing the residue was reweighed. Water absorption capacity (WAC) and oil absorption capacity (OAC) were calculated using equations 4.2 and 4.3, respectively.

$$\text{WAC } (\text{ml/g}) = \frac{W_2 - W_1}{W} \qquad \text{Equation 4.2}$$

$$\text{OAC } (\text{ml/g}) = \frac{W_2 - W_1}{W} \qquad \text{Equation 4.3}$$

Where, W = Weight of dry sample, W_1 = Weight of the tube plus the dry sample, W_2 = Weight of tube plus wet sample

4.3.2 Swelling capacity analysis of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

The swelling capacity (SC) was determined using the method described by Adeleke *et al* (2017). Samples of BGNPI, MOLPI, and BAMOLP (1 g) were separately dispersed in 10 ml distilled water in separate pre-weighed 50 ml centrifuge tubes. The mixture was heated in a temperature regulated-water bath at a constant temperature of 60, 70, 80, and 90^{o}C for 15 min, cooled to room temperature, and centrifuged at 5300 rpm for 10 min. The SC was expressed as a percentage increase in sample weight.

4.3.3 Foaming capacity and stability analysis of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

The foaming characteristics of the protein isolates were investigated using the method of Adebowale *et al.* (2011). Thirty ml of the protein isolate suspension (1 g/100 g, adjusted to the required pH 2-10) in a 50 ml centrifuge tube was blended in a polytron homogenizer (IKA T18, Ultra-Turrax, Bechthai, Bangkok, Thailand), at 12,000 rpm for 10 min at room temperature. The volume of foam (ml) that was present above the surface of the liquid contained in a 50 ml centrifuge tube was measured. Foam expansion and foam stability were calculated by equations 4.4 and 4.5, respectively.

$$\text{Foam expansion (\%)} = \frac{\text{Vol after whipping- Vol before whipping}}{\text{Vol before whipping}} \times 100 \qquad \text{Equation 4.4}$$

$$\text{Foaming Stability (\%)} = \frac{\text{Foaming volume after time t}}{\text{initial foam volume}} \times 100 \qquad \text{Equation 4.5}$$

4.3.4 Emulsifying capacity and stability determination of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

Emulsifying capacity and stability were determined by the method reported by Lawal *et al*. (2007) and Arise *et al.* (2015) with slight modification. A 0.5 g sample of BGNPI, MOLPI, and BAMOLP were dispersed in 50 ml Millipore water in separate 100 ml beakers. Five millilitres (5 mL) portions of protein solution were homogenised with 5 mL of sunflower oil and the mixture was vortexed for 10 min. The emulsions were centrifuged at 3200 rpm for 5 min. The height of the emulsified layer and that of the total content in the tube was measured. The emulsifying activity (EA) and stability were calculated using equation 4.6

$$\text{EA (\%)} = \frac{\text{height of emulsified layer in the tube}}{\text{height of the total content in the tube}} \qquad \text{Equation 4.6}$$

Emulsion stability was determined by heating the emulsion at 80°C for 30 min after which it was centrifuged at 3200 rpm for 5 min. The height of the emulsified layer and that of the total content in the tube after heating were measured. The emulsion stability was calculated using equation 4.7

$$\text{ES (\%)} = \frac{\text{height of emulsified layer after heating}}{\text{height of the emulsified layer before heating}} \qquad \text{Equation 4.7}$$

4.3.5 Protein solubility determination of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

Effect of pH (2-11) on the solubility of the isolates and their complex was investigated using the method described by Kudre *et al*. (2013a) with modification. Protein solubility (%) was investigated by dissolving 0.3 g BGNPI, MOLPI, BAMOLP complex each in 30 ml distilled water. The pH was adjusted to different values (2–11) with 0.5 N HCl or NaOH, and it was stirred at room temperature for 1 h. Thereafter, the mixture was checked, adjusted to the testing pH, and was centrifuged at 800 x g for 10 min. The supernatant was freeze-dried and tested for protein using a Truspec Nitrogen Analyser (LECO-Laboratory Equipment, USA). Protein solubility (%) was determined as in equation 4.8

$$\% \text{ Protein solubility} = \frac{\text{Protein in the supernatant}}{\text{Total protein in the isolate}} \times 100\ \% \qquad \text{Equation 4.8}$$

4.4 The proximate and amino acid profile

BGNPI, MOLPI, and BAMOLP were subjected to proximate analyses. Protein determination by Truspec Nitrogen Analyser (LECO-Laboratory Equipment), total fat according to AOAC (2005) method 996.06, moisture and ash according to AOAC (2005) method 934.01 and 923.03, respectively. The carbohydrate was determined by difference.

The amino acid in BGNPI, MOLPI, and BAMOLP was determined using the Waters Acquity Ultra performance liquid chromatography (UPLC) fitted with a photodiode array detector. The amino acid was derivatised and separated with UV detection using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). The derivatisation procedure was as follows: Borate buffer (70 µl) was pipetted into a vial, and mixed with 10 µl of the diluted samples. Waters Accq. Tag Ultra Reagent (AQC reagent) (20 µl) was added to the mixture. The vial was capped and vortexed to achieve effective mixing. The vial was incubated at 55^{o}C for 10 minutes. After 10 minutes the samples were placed on an autosampler tray for analysis.

4.5 Rheological properties of protein isolates and complex

The method adopted by Adeyi *et al*. (2014) was used to evaluate the rheological properties of BGNPI, MOLPI, and BAMOLP. The rotational test was conducted to determine the flow behaviour of BGNPI, MOLPI, and BAMOLP using a discovery Hybrid rheometer with a concentric cylinder geometry (DIN rotor and cup with specifications: bob diameter: 28 mm; bob length 42 mm; cup diameter 30.4 mm). The protein samples and complex were separately transferred to the rheometer plate. The steady-state test was carried out by subjecting samples under a constant shear rate of 100 s^{-1} for 10 min. Thereafter, the shear rate was varied from 10^{-2} to 1000 s^{-1} at 25^{o}C. The apparent viscosity was obtained from the relationship between the shear stress versus shear rate. The aim was to describe the variation in the rheological

properties of BGNPI, MOLPI, and BAMOLP under steady shear; measurement was carried out in triplicate.

4.6 Statistical analysis

All experiments were conducted in triplicate. To determine mean differences between treatments, obtained data were subjected to multivariate analysis of variance (ANOVA). Where differences existed, separation of means was carried out using Duncan's multiple range test (IBM SPSS, version 25). Application of Kruskal Wallis test (non-parametric ANOVA) where normality test was violated.

4.7 Results and Discussion

4.7.1 Physical characteristics of Bambara groundnut and *Moringa oleifera* leaf protein isolates and their complex

1 *Physical appearance and Colour characteristics of Bambara groundnut and Moringa oleifera leaf protein isolates and their complex*

The physical appearance of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* leaf protein isolate (MOLPI), and their complex (BAMOLP) are presented in Figure 4.2.

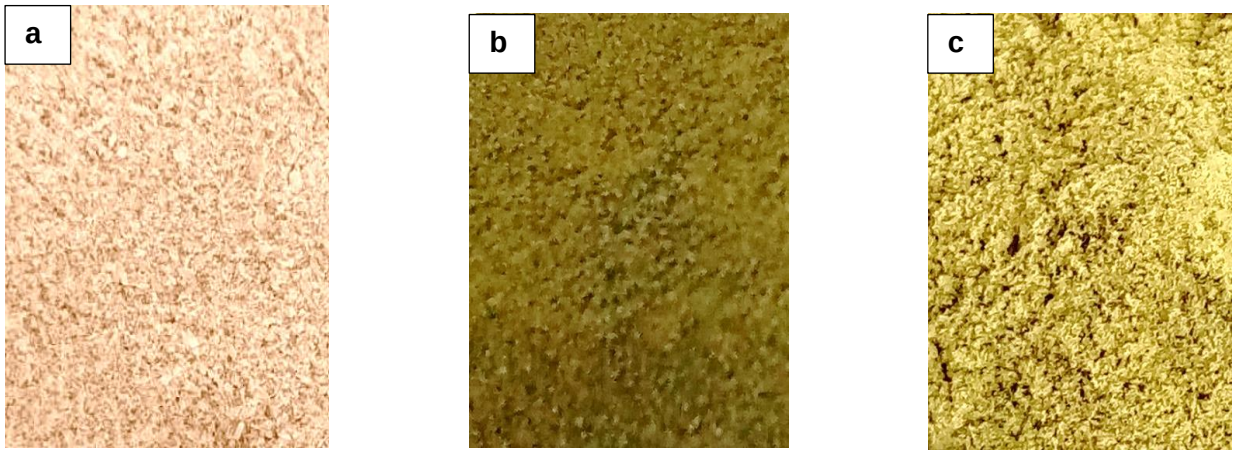

Figure 4.2 Physical appearance of (a) Bambara groundnut protein isolate (BGNPI), (b) *Moringa oleifera* protein isolate (MOLPI), and (c) Bambara groundnut-*Moringa oleifera* leaf protein complex (BAMOLP).

The descriptive for colour characteristics of BGNPI, MOLPI, and BAMOLP are presented in Table 4.1. The average values of lightness for BGNPI, MOLPI, and BAMOLP were 63.38, 34.09, and 49.90, respectively. Both isolate and their complex differ significantly ($p < 0.05$) in lightness, BGNPI being significantly lighter than the MOLPI and BAMOLP. The redness of BGNPI, MOLPI, and BAMOLP had mean values of 6.62, 0.19, and 0.14, respectively. The redness of MOLPI and BAMOLP did not differ significantly ($p > 0.05$). However, the redness of BGNPI differed significantly ($p < 0.05$) compared to MOLPI and BAMOLP. The yellowness and chroma of the protein isolates (BGNPI, MOLPI) and their complex (BAMOLP) differ significantly ($p < 0.05$). There was no significant difference in the hue angle of MOLPI (89.44^{o}) and BAMOLP (89.66^{o}) but the hue angle of BGNPI (72.69^{o}) differs significantly ($p < 0.05$) compared to MOLPI and BAMOLP. The protein isolates and their complex are yellowish-red, the intensity of the colour is low because the chroma values were low, the lower the chroma, the less intense the colour. BGNPI has the lightest colour, followed by BAMOLP while MOLPI has a darker colour.

BGNPI is lighter, redder, yellower, and more saturated than MOLPI, but had a lower hue compared to MOLPI and BAMOLP. The lightness and redness of BGNPI are higher compared to BAMOLP but lower in yellowness, less saturated, and has a lower hue. MOLPI has the least of the colour attributes measured. BGNPI can be described as a light yellowish-red material while MOLPI and BAMOLP are dark yellowish materials. Although *Moringa oleifera* leaf powder (MOLP) which is the starting material for MOLPI is green, spectrophotometry measurement indicated a dark yellowish material. The colour change may be due to the impact of heat during the extraction process of MOLPI and the oven drying process. The yellowness of BGNPI, MOLPI, and BAMOLP can be ascribed to phenolic compounds associated with redness and yellowness which includes anthocyanins, quercetin, cynanides, as well as β-carotene, a colour pigment responsible for red, yellow, and orange colour in plants (Delgado-Vargas *et al*., 2000; Cheynier, 2005; Dai and Mumper, 2010; Dragović-Uzelac *et al*., 2010).

The colour difference (Δ E) between BGNPI and MOLPI, MOLPI and BAMOLP, and BGNPI and BAMOLP were determined to be 30.08, 16.45, and 15.14, respectively. The colour differences are perceivable since a colour difference of 1 is just noticeable.

Table 4.1 Colour characteristic of BGNPI, MOLPI, and BAMOLP[1,2]*

Protein isolates	**Colour Values**				
	L*	a*	b*	C*	h^o
BGNPI	63.38 ± 0.31[a]	6.62 ± 0.09[a]	21.24 ± 0.31[a]	22.25 ± 0.33[a]	72.69 ± 0.05[a]
MOLPI	34.09 ± 0.50[c]	0.19 ± 0.07[b]	18.94 ± 0.39[b]	18.95 ± 0.38[b]	89.44 ± 0.19[b]
BGNPI-BAMOLP	49.90 ± 0.15[b]	0.14 ± 0.01[b]	23.51 ± 0.45[c]	23.51 ± 0.45[c]	89.66 ± 0.01[b]

[1]BGNPI: BGN protein isolate, MOLPI: *Moringa oleifera* leaf protein, BAMOLP: BGN-*Moringa oleifera* complex. Mean values ± standard deviation of triplicate determination.

[2]Mean values in the same column followed by different letters are significantly ($p < 0.05$) different; L*: Lightness; a*: Redness: b*: Yellowness; C*: Chroma, h^o: Hue angle, ΔE: Colour difference using L*C*h* and CIE-L*a*b* and colour space system.

The observed colour differences can be attributed to differences in the colour pigment of BGN and MOLP which are the starting materials.

2 *Particle morphology of Bambara groundnut and Moringa oleifera leaf protein isolates and their complex by scanning electron microscopy (SEM)*

Scanning electron micrograph (SEM) of BGNPI, MOLPI, and BAMOLP are shown in Figure 4.3. The SEM images were obtained to examine the particle morphology of the protein isolates and their complex. BGNPI exhibited a less collapsed structure, with a partially solid surface, having dents (shown by the yellow arrow) and a slightly dimpled cracked surface with irregular shape (shown by the red arrow). MOLPI and BAMOLP showed a collapsed structure with

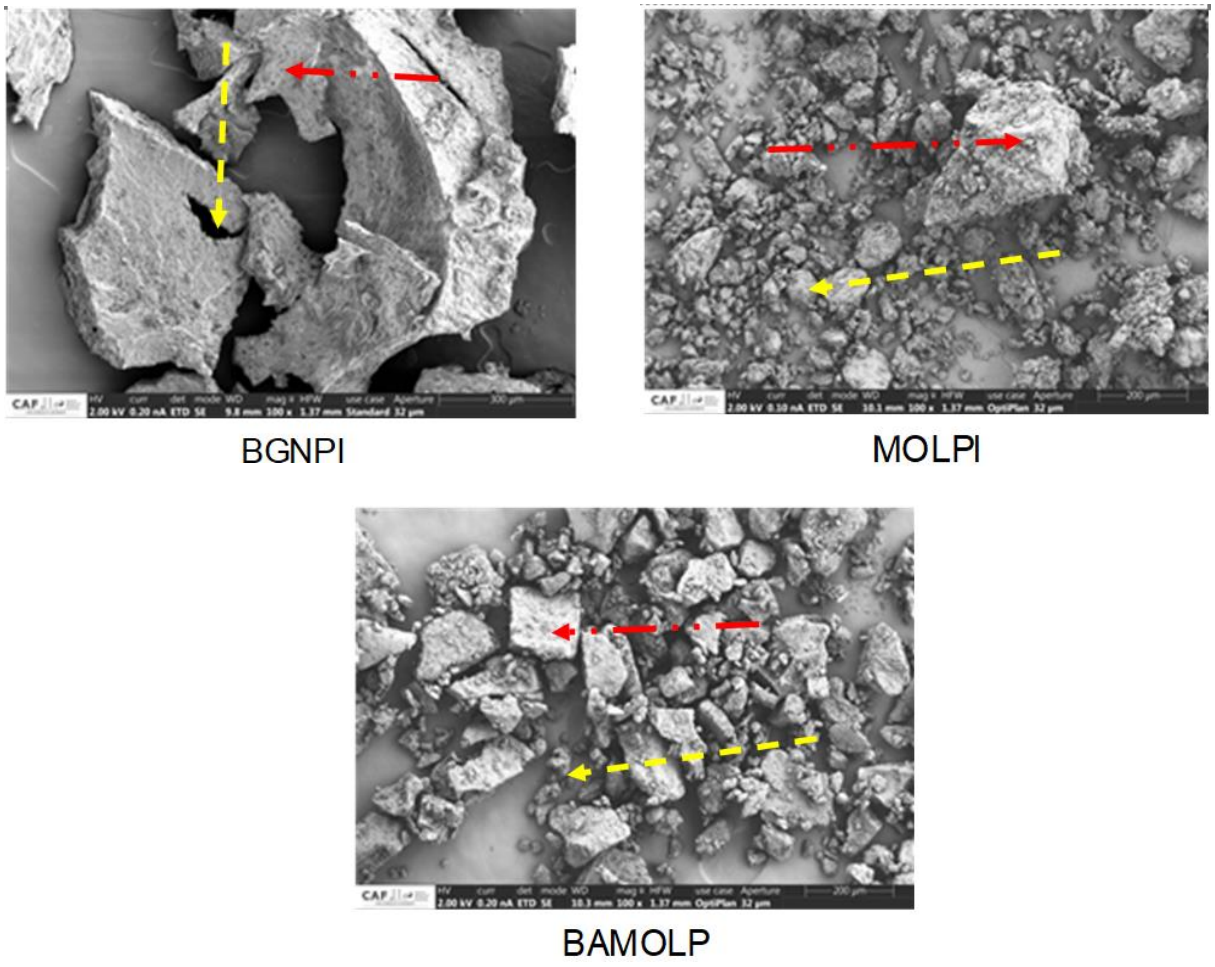

Figure 4.3 Scanning Electron Micrograph of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* protein isolate (MOLPI) and their complex (BAMOLP), (magnification x200)

clumped agglomerated layers as shown by the red arrows, rough surface, and irregular shape (shown by the yellow arrows). The isolates and their complex showed larger particle sizes at lower magnification (x 100). The cracking surface observed for BGNPI is comparable to the result obtained for black and white Bambara groundnut protein reported by Kaptso *et al.* (2015). Irregular shapes with different sizes were also reported for BGN fibre (Diedericks, 2014).

3. *Water Activity of Bambara groundnut and Moringa oleifera leaf protein isolates and their complex*

Water activity (a_w) of BGNPI, MOLPI, and BAMOLP were 0.18, 0.68, and 0.37 respectively. The a_w of a food refers to the water content available for microbial growth.and it is a major factor in controlling the rate of food spoilage (Fontana & Cambell, 2004). Water in a food product is either free or bound. Free water is found in pores of the food material and can be released when the food is pressed. Bound water describes water that is not affected by heat treatment of food, it exhibits no vapour and cannot be frozen below 0^{o}C. Deterioration of food due to microbial growth (moulds, yeast and pathogens) happens at a range of 0.6 to 1.0 (Roos, 2001; Fontana & Cambell, 2004). However, some enzymatic reactions, such as browning occurs at a range of 0.3 to 1.0 and increases rapidly at a_w 0.6 to 0.8. BGNPI had the lowest a_w of 0.18, followed by BAMOLP with a_w of 0.37 and MOLPI had a_w of 0.68. BGNPI and BAMOLP had a_w less than 0.6 and are therefore less prone to microbial spoilage but a_w of MOLPI is at the boundary in which microbial spoilage and enzymatic reactions start, and may therefore be more susceptible (Buerman *et al.*, 2019). The high a_w of MOLPI may be due to the drying method utilized, it was oven-dried in a dehydrator at 50^{o}C for 8 hours while BGNPI was freeze-dried. Freeze drying may be a better alternative for drying MOLPI rather than oven-drying to ensure sufficient removal of water from the product, and consequently achieve lower water activity. The freeze-drying involves dehydration by sublimation and products are sufficiently and uniformly dried, while oven drying is done by evaporation, in which there is a probability of moisture retention and even hardening of the product may occur at high temperature (Ahmed & Al-Attar, 2015).

4.7.2 Proximate composition of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* leaf protein isolate (MOLPI) and BGN-Moringa protein complex.

The chemical composition of BGNPI, MOLPI, and BAMOLP are shown in Table 4.2. The moisture content of MOLPI (9.57%), is significantly ($p < 0.05$) higher than that of BGNPI (2.61%) and BAMOLP (4.70%). The moisture content of BGNPI is lower than that reported by Kudre (3.16%). BGNPI, MOLPI, and BAMOLP contained 61.09, 39.42 and 63.51% protein, respectively and are significantly ($p < 0.05$) different. Factors responsible for variation in the

Table 4.2 Chemical composition of BGNPI, MOLPI, and BGNMPC (g/100 g)[1,2]

Protein isolate	Proximate (g/100 g)				
	Moisture	**Ash**	**Fat**	**Protein**	**Carbohydrate**
BGNPI	2.6 ± 0.1[a]	1.60 ± 0.0[a]	11.5 ± 0.4[a]	61.1 ± 1.3[a]	24.1 ± 0.6[a]
MOLPI	9.6 ± 0.1[b]	7.09 ± 0.0[b]	2.2 ± 0.2b	39.4 ± 0.5[b]	51.3 ± 0.7[b]
BAMOLP	4.7 ± 0.2[c]	2.42 ± 0.0[c]	8.8 ± 0.3[c]	63.5 ± 1.1[c]	25.3 ± 0.9[a]

[1]BGNPI: BGN protein isolate, MOLPI: *Moringa oleifera* leaf protein, BAMOLP: BGN-*Moringa oleifera* complex.

[2]Mean values are triplicate determinations ± standard deviation. Means within a column followed by different superscripts are significantly ($p \leq 0.05$) different.

protein contents of the isolates could vary from the actual content of protein in the raw materials to the extraction method. The Bambara groundnut flour (BGNF) and *Moringa oleifera* (MO) leaf powder used for the extraction of the protein isolates contained 16.8 and 31.8% protein, respectively. Moreover, for BGNPI, whole flour was used for the extraction, and the deffating process was mild (80% ethanol) because the isolates will be used as a functional ingredient in a food product. There is therefore the possibility that other components such as polyphenols co-precipitated at pH 5 during protein recovery and extraction of the isolate.

The BGNPI protein content of 61.1% (Table 4.1) obtained in this study is lower than 86.0% reported for BGNPI by Eltayeb *et al.* (2011). The difference could be due to the absence of fat in the isolate obtained by the method of Eltayeb (2011). The fat was removed by n-hexane in a soxhlet apparatus. Hexane was not appropriate for defatting in this work because the protein isolate will be applied in a food product. Therefore, 80% ethanol was used and fat was not completely removed. The content of protein, 39.0% in MOLPI is comparable to 39.0% reported by Sodamade *et al* (2013) in which a similar method of extraction was used and higher than 19.94% reported by Ahmed (2016) for *Moringa oleifera* leaf protein concentrate in which isoelectric precipitation was employed. In this study, aqueous extraction with heat treatment was used for extracting MOLPI. There is no literature record to compare with Bambara groundnut-*Moringa oleifera* protein complex (BAMOLP), nevertheless, it can be compared to BGNPI, since BAMOLP was produced with ratio 2:9 of MOLPI and BGNPI, it is therefore expected that the chemical composition, functional properties, and structural behaviour of BGNPI and BAMOLP should be similar. The similarity in the chemical composition of BGNPI and BAMOLP is confirmed in this study (Table 4.2).

The fat contents of BGNPI, MOLPI, and BAMOLP were 11.5, 2.2, and 8.8%, respectively, and are significantly different ($p < 0.05$). The fat content of BGNPI is higher than 3.2% reported by Kudre *et al* (2013). It is possible that fat had undergone saponification at the

alkaline pH with NaOH used for solubilisation, at the isoelectric point (pH 4.5), and precipitated together with the protein. The defatting may have been insufficient.

Ash content was 1.60, 7.09, and 2.42% for BGNPI, MOLPI, and BAMOLP, respectively, and they were significantly different ($p < 0.05$). The ash content of BGNPI (3.11%) is lower than reported by Kudre (2013) for Bambara groundnut protein isolate. The ash content of MOLPI (7.09%) indicates that MOLPI had higher mineral content compared to BGNPI and BAMOLP. This could be due to higher content in the added *Moringa oleifera* leaf powder compared to the ash content of Bambara groundnut flour (Fasuyi and Aletor, 2005; Busani *et al.*, 2011; Alain Mune Mune *et al.*, 2016), but it is lower than 9.30% reported by Ahmed (2016) for *Moringa oleifeira* leaf protein concentrates. The carbohydrate content of BGNPI (24.07%) and BAMOLP (25.32%) are not significantly different but MOLPI (51.29%) was significantly higher ($p < 0.05$) compared to BGNPI (24.07%) and BAMOLP (25.32%). The carbohydrate content of BGNPI is higher compared to 5.48% reported by Kudre (2013).

4.7.3 Amino acid composition of Bambara groundnut protein isolates, *Moringa oleifera* leaf protein isolates, and Bambara groundnut–*Moringa oleifera* leaf protein complex

The amino acid composition of BGNPI, MOLPI, and BAMOLP are presented in Table 4.3. The total amino acids in BGNPI, MOLPI, and BAMOLP were 75.11, 50.00, and 71.83 g/100 g, respectively. The essential amino acid of the protein isolates were 39.83, 47.88, and 40.83% of the total amino acids, respectively. Bambara groundnut protein isolate showed higher histidine, lysine, leucine, valine, isoleucine, and phenylalanine in comparison to MOLPI while MOLPI showed higher threonine and methionine compared to BGNPI (Figure 4.3). Tryptophan was lost during acid hydrolysis of the protein, hence it was absent in all the samples. Cysteine was not detected in this study, which is comparable to the study of Adebowale *et al* (2011), Kudre *et al* (2013), and Arise *et al* (2017) for BGN isolate. Destruction of cysteine, threonine, arginine, serine, and lysine occurs by alkali processing (Zhu *et al.*, 2006), hence, cysteine might have been lost during processing. The total amino acid (75.11 g/100 g) obtained in this study for BGNPI is lower than 81.07 g/100 g reported by Kudre *et al* (2013) for whole BGN and also lower than 90 and 89.76 g/100 g reported by Adebowale *et al* (2011) for white and brown Bambara groundnut.

Methionine content of BGNPI and MOLPI was 1.77 and 2.50 g/100 g, respectively. These values are appreciable since methionine is usually deficient in legumes and green leaves (Fasuyi and Aletor, 2005; Duranti, 2006; Hillocks *et al.*, 2012; Kudre *et al.*, 2013). MOLPI was richer in methionine than BGNPI. Therefore, the aim of selecting MOLPI to form a complex with BGNPI with higher methionine content was successful. The content of methionine 1.77 g/100g obtained in this study for BGNPI was higher than 1.27 g/100g reported by Kudre *et al* (2013) for BGN protein. Importantly, methionine 1.86 g/100 g in BAMOLP is

higher than 0.3 g/100 g in soya bean and comparable to 2.5 g/100 g recommended by FAO/WHO/UNU (Adam *et al.*, 2015). BGNPI, MOLPI, and BAMOLP are good sources of

Table 4.3 Amino acid composition BGNPI, MOLPI, and BAMOLP[1,2]

	Protein isolate amino-acid (g/100g)		
Essential Amino acid	**BGNPI**	**MOLPI**	**BAMOLP**
Threonine	3.02 ± 0.02$^{a.}$	3.44 ± 0.48^{b}	2.85 ± 0.04^{a}
Methionine	1.77 ± 0.00^{a}	2.50 ± 0.08^{c}	1.86 ± 0.03^{b}
Phenylalanine	9.41 ± 0.05^{a}	5.80 ± 0.12^{c}	6.55 ± 0.11^{b}
Histidine	2.85 ± 0.07^{a}	1.29 ± 0.02^{c}	1.71 ± 0.00^{b}
Lysine	3.53 ± 0.10^{a}	2.03 ± 0.18^{c}	4.49 ± 0.23^{b}
Valine	3.31 ± 0.20^{a}	2.75 ± 0.13^{b}	3.21 ± 0.02^{c}
Isoleucine	3.02 ± 0.20^{a}	2.11 ± 0.07^{c}	2.75 ± 0.08^{b}
Leucine	6.21 ± 0.16^{a}	4.02 ± 0.10^{c}	5.91 ± 0.10^{b}
Non-Essential Amino acid (g/100 g)			
Serine	5.05 ± 0.24^{a}	2.79 ± 0.65^{c}	4.41 ± 0.20^{b}
Arginine	6.31 ± 0.35^{a}	3.69 ± 0.32^{c}	5.33 ± 0.12^{b}
Glycine	2.65 ± 0.10$^{a.b}$	2.62 ± 0.12$^{a.b}$	2.49 ± 0.09^{c}
Asparagine	7.55 ± 0.35^{a,b}	3.37 ± 0.26^{c}	7.95 ± 0.61^{b}
Glutamine	12.90 ± 0.39^{a}	4.53 ± 0.11^{b}	13.11 ± 0.66^{a}
Alanine	3.12 ± 0.03^{a}	3.01 ± 0.15^{a}	3.11 ± 0.02^{a}
Proline	2.87 ± 0.02^{a}	2.36 ± 0.26^{b}	2.70 ± 0.00a^{c}
Tyrosine	4.74 ± 0.11^{a}	3.69 ± 0.33^{b}	3.40 ± 0.13^{b}
TAA	75.11	50.00	71.83
TEAA	29.92	23.94	29.33
TNAA	45.19	26.06	42.50
%TEAA/TAA	39.83	47.88	40.83
%TNAA/TAA	60.17	52.12	59.17

[1]MOLPI: *Moringa oleifera* leaf protein isolate; BGNPI: Bambara groundnut (BGN) protein isolate; BAMOLP: BGN and *Moringa oleifera* leaf protein complex.

[2]Mean values of triplicate determinations ± standard deviation. Means within the same row with different superscripts differ significantly ($p < 0.05$)

arginine, a conditionally essential amino acid with BGNPI having the highest 6.31 g/100 g, while BAMOLP and MOLPI have 5.33 and 3.39 g/100 g respectively.

The high lysine content of the BGNPI, MOLPI, and BAMOLP is a boost to their nutritional value, which will promote their use as a supplementary protein in the formulation of cereal products that are deficient in lysine. BAMOLP had the highest content of lysine, 4.49%.

The non-essential amino acid of BGNPI, MOLPI, and BAMOLP was 60.17, 52.12, and 59.17% of the total amino acids, respectively. Major non-essential amino acids observed were glutamine and asparagine. These non-essential amino acids were higher in BAMOLP, BGNPI, and MOLPI. Arginine has been reported to prevent heart disease (Adebowale *et al.*, 2011).

Amino acids are organic compounds, which are precursors of proteins; therefore, they influence the quantity and quality of protein (Fasuyi and Aletor, 2005; Moyo *et al.*, 2011; Alain Mune Mune *et al.*, 2016). Amino acids are categorised as essential and non-essential and vary according to animal species and their production system. They are indispensable in the production of enzymes, immunoglobins, hormones, growth, and repair of body tissues and form the structure of red blood cells (Brisibe *et al*., 2009). Furthermore, they play an important role in the formation of glucose, acting as a buffer when other precursors are in short supply. Amino acids are essential for the performance of specific functions in the body (Moyo *et al.*, 2011). BAMOLP is, therefore, a good source of essential amino acids, since it is especially higher in methionine, phenylalanine, and histidine compared to whey, pea, brown rice, soy, hemp, and wheat protein (Figure 4.4). The amino acid content of BAMOLP is higher in threonine, phenylalanine, lysine, and leucine when compared to FAO/WHO reference pattern (Adam *et al.*, 2015). BAMOLP will be a suitable functional food ingredient in value-added products.

4.7.4 Functional properties of Bambara groundnut protein isolates, *Moringa oleifera* leaf protein isolates, and Bambara groundnut-*Moringa oleifera* leaf protein complex

The descriptive statistics for the functional properties of Bambara groundnut protein isolates (BGNPI), *Moringa oleifera* leaf protein isolates (MOLPI), and Bambara groundnut-*Moringa oleifera* leaf protein complex is presented in Table 4.4. The solubility of the isolates and complex is also shown in Tables 4.5 and 4.6.

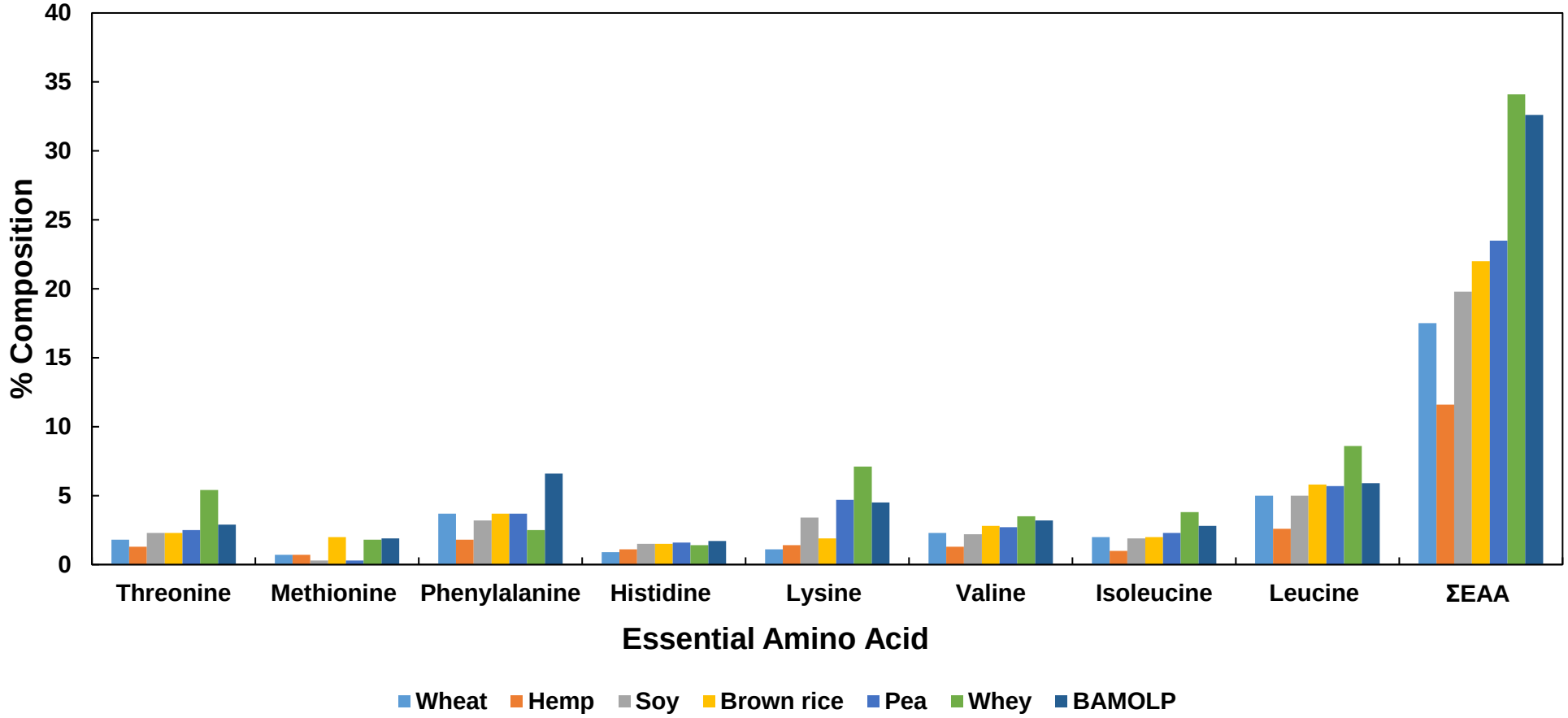

Figure 4.4 Comparison of amino acids in Bambara groundnut protein complex and other protein sources. BAMOLP: BGN and Moringa protein complex; ΣEAA: Total essential amino acid

.

1 *Oil absorption capacity*.

The oil absorption capacity (OAC) of BGNPI, MOLPI, and BAMOLP was 2.26, 0.89, and 0.95 g/g, respectively as shown in Table 4.4 and are significantly ($p < 0.05$) different. BGNPI exhibited the highest OAC and it is higher than 1.04 g/g reported by Adeleke *et al*. (2017) for Bambara groundnut protein concentrates. The OAC for MOLPI obtained in this study is lower than 3.87 g/g reported by Ahmed (2016) for *Moringa oleifera* leaf protein concentrates. Differences in the oil binding capacity of the isolates may be due to variations in the presence of non-polar side chains, which might bind the hydrocarbon side chains of oil among the isolates. Oil absorption capacity describes the ability of fat to bind the non-polar side chains of protein. The isolates will be suitable in food application especially in flavour retention, improvement of palatability, and extension of shelf life particularly in bakery or meat products where fat absorption is anticipated.

Table 4.4 Functional properties of BGNPI, MOLPI, and BAMOLP[1]

Parameter	**BGNPI**	**MOLPI**	**BAMOLP**
OAC g/g	2.26 ± 0.15^{a}	0.89 ± 0.04^{c}	0.95 ± 0.03^{b}
WAC g/g	1.31 ± 0.02^{a}	1.5 ± 0.03^{b}	1.22 ± 0.03^{c}
Emulsifying Capacity %	39.17 ± 4.25^{a}	45.83 ± 0.00^{b}	50 ± 0.00^{b}
Emulsifying Stability %	56.36 ± 5.53^{a}	47.28 ± 3.04^{b}	56.37 ± 3.15^{a}

[1]BGNPI: Bambara groundnut protein isolate, MOLPI: *Moringa oleifera* leaf protein isolate, BAMOLP: Bambara groundnut-*Moringa* protein complex, OAC: Oil absorption capacity, WAC: water absorption capacity.

2. *Water absorption capacity*

The water absorption capacity for BGNPI, MOLPI, and BAMOLP was 1.31, 1.5, and 1.22 g/g, respectively (Table 4.4), and are significantly ($p < 0.05$) different. The value of WAC obtained in this study for *Moringa oleifera* leaf protein isolate 1.13 g/g is lower than 5.82 g/g reported by Ahmed (2016) for *Moringa oleifera* leaf protein concentrates. Disruptions of protein happen when isoelectric precipitation is employed in the extraction of protein, causing a limitation in the interaction between the protein and the surrounding aqueous system, thereby reducing WAC. Isoelectric precipitation was used for the extraction of BGNPI, which may be the reason for the low value of WAC obtained in this study. Water absorption capacity is the ability of flour to absorb water, swell and thus enhance the consistency of food products.

Effect of temperature on WAC of BGNPI, MOLPI, and BAMOLP is presented in Figures 4.5. The WAC of the isolates increased with an increase in temperature from 60 to 90^{o}C.

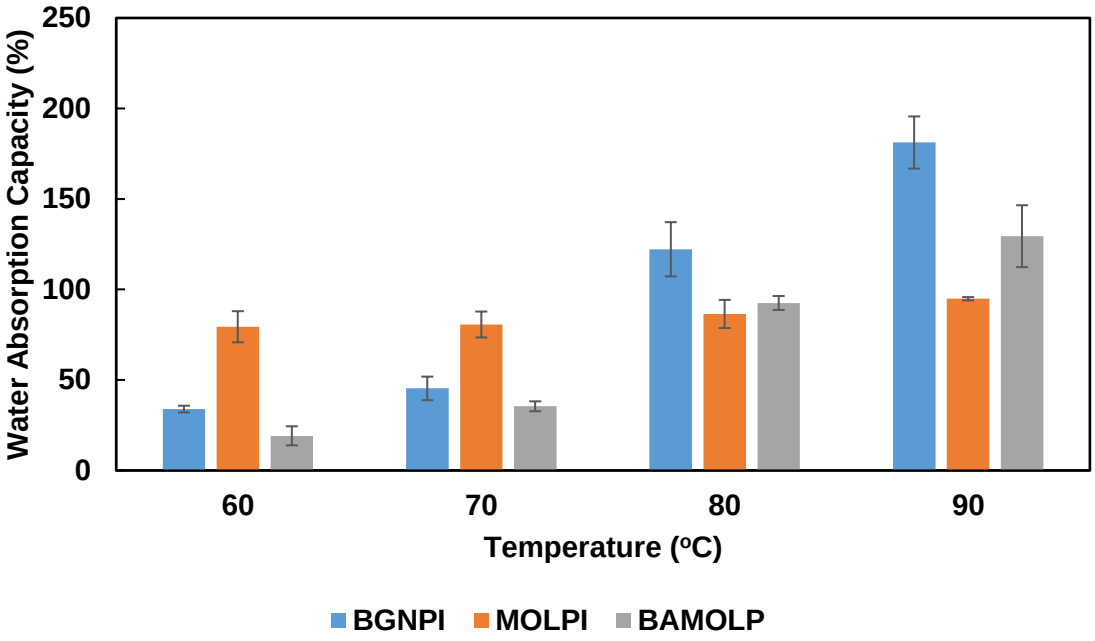

Figure 4.5 Effect of temperature on water absorption capacity (%) of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* protein isolate (MOLPI), and BGN and Moringa protein complex (BAMOLP). Error bars are the standard deviation of the mean.

BGNPI has the highest capacity to absorb water with the utmost value of 181.19% at 90ºC. The isolates can be used as thickeners in the food system because they exhibit high WAC, which improves consistency.

The swelling capacity of isolates increased significantly with an increase in temperature from 60 to 90ºC (Figure 4.6), a similar observation was reported by Adeleke *et a*l. (2018). Factors that can influence swelling capacity are the nature of the material, type of treatment, process conditions, and differences in the molecular organization within the granules of the sample. Higher swelling capacity at 90ºC implies that elevated temperature promotes the penetration of water into the protein granules (Adeleke *et al.*, 2018). Food ingredients that have high swelling capacity are advantageous in the manufacture of confectionery, BGNPI, MOLPI, and BAMOLP can be used in such applications.

3. *Emulsifying capacity and stability*

Emulsifying capacity (EC) of BGNPI, MOLPI, and BAMOLP was 39.2, 45.8, and 50.0%, respectively as presented in Table 4.4. The emulsifying capacity of BGNPI was significantly ($p < 0.05$) lower than the emulsifying capacity of MOLPI and BAMOLP. There was no significant difference between the emulsifying capacity of MOLPI and BAMOLP. The EC is an indication of the ability of a protein to absorb rapidly at the water-oil interphase during the formation of

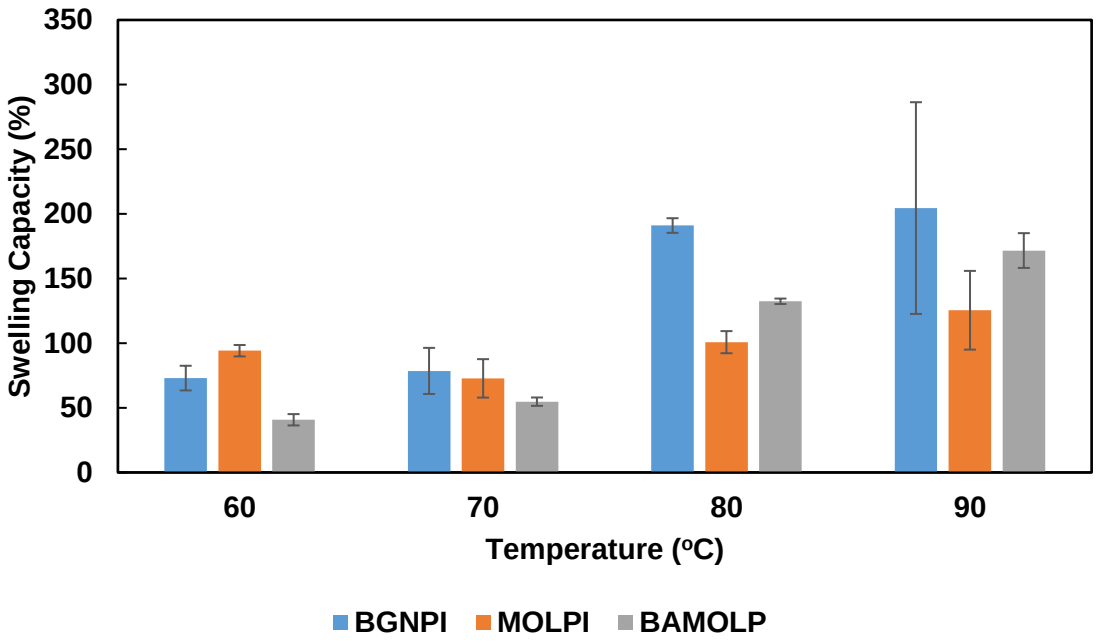

Figure 4.6 Effect of swelling capacity (%) of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* protein isolate (MOLPI), and BGN and Moringa protein complex (BAMOLP). Error bars are the standard deviation of the mean.

emulsion to prevent flocculation and coalescence. This result indicates that MOLPI and BAMOLP have a higher capacity to reduce the migration rate of oil droplets and particle size. Emulsifying stability (ES) was 56.4, 47.3, and 56.4%, respectively (Table 4.4). There was no significant difference between the emulsifying stability of BGNPI and BAMOLP, however, MOLPI is significantly ($p < 0.05$) different. Emulsifying stability unveils the ability of the samples to maintain a stable emulsion over a long time by preventing the flocculation and coalescence of the oil globules (Tangsuphoom & Coupland, 2008; Du *et al.*, 2014). BAMOLP and BGNPI have the highest emulsifying stability (56.4%) while MOLPI has the lowest (47.3%) emulsifying capacity

4 *Effect of pH on foam capacity and stability*

The effect of pH on the foam capacity of BGNPI, MOLPI, and BAMOLP is presented in Figure 4.7. Foam capacity is the ability of a solution to produce stable foam. The foam capacity of BGNPI, MOLPI, and BAMOLP was observed to be pH-dependent. BGNPI and BAMOLP exhibited the highest foaming capacity at acidic pH 2 while it was alkaline (pH 10) for MOLPI.This observed difference might be due to an increase in solubility of protein at alkaline

and acidic pH, as well as structural rearrangement of protein as a result of denaturation. The least foam capacity was at pH 4 for all the protein samples. Ahmed (2016) reported a similar

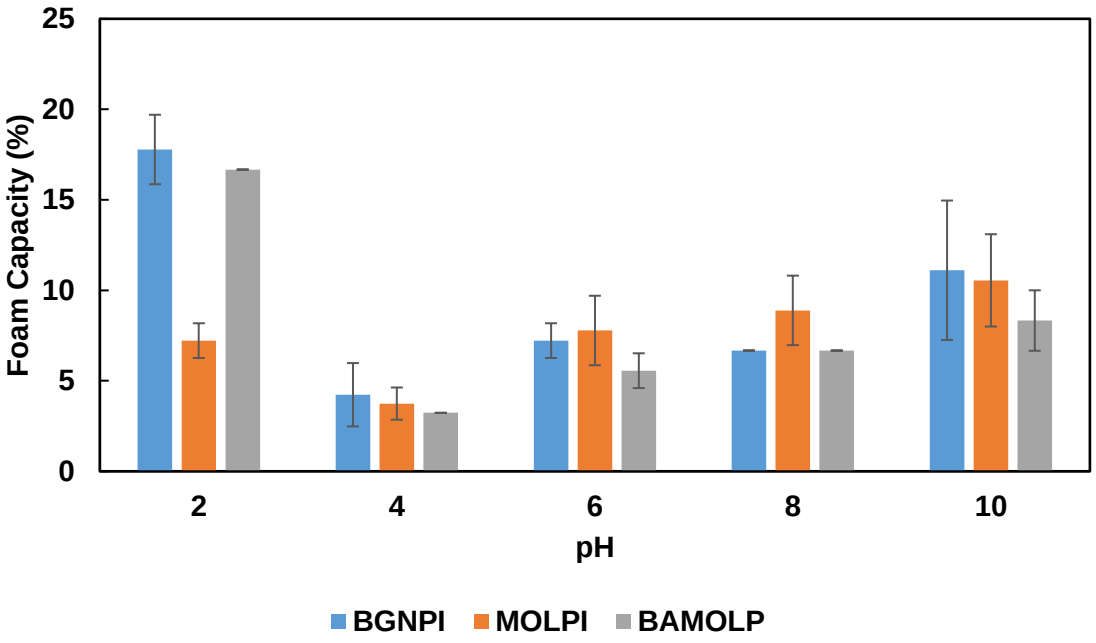

Figure 4.7 Foam Capacity (%) of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* protein isolate (MOLPI), and BGN and Moringa protein complex (BAMOLP). Error bars are the standard deviation of the mean.

result for the foaming capacity of *Moringa oleifera* leaf protein concentrate, where improved foaming capacity was observed at alkaline pH.

The highest foam stability occurred at pH 8 and 10 for BGNPI, at pH 2, 8, 10 for MOLPI, and pH 2 for BAMOLP (Figure 4.8). The least foam stability occurred at pH 4 for BGNPI, pH 6 for MOLPI, and pH 10 for BAMOLP. The differences in the foaming properties of the proteins BGNPI, MOLPI, and BAMOLP may be due to the level of protein present in their flour and the degree of denaturation. The protein and emulsifier present in the solution impact foam texture and are essential in retaining and improving the stability of foam (Blasco *et al.*, 2011).

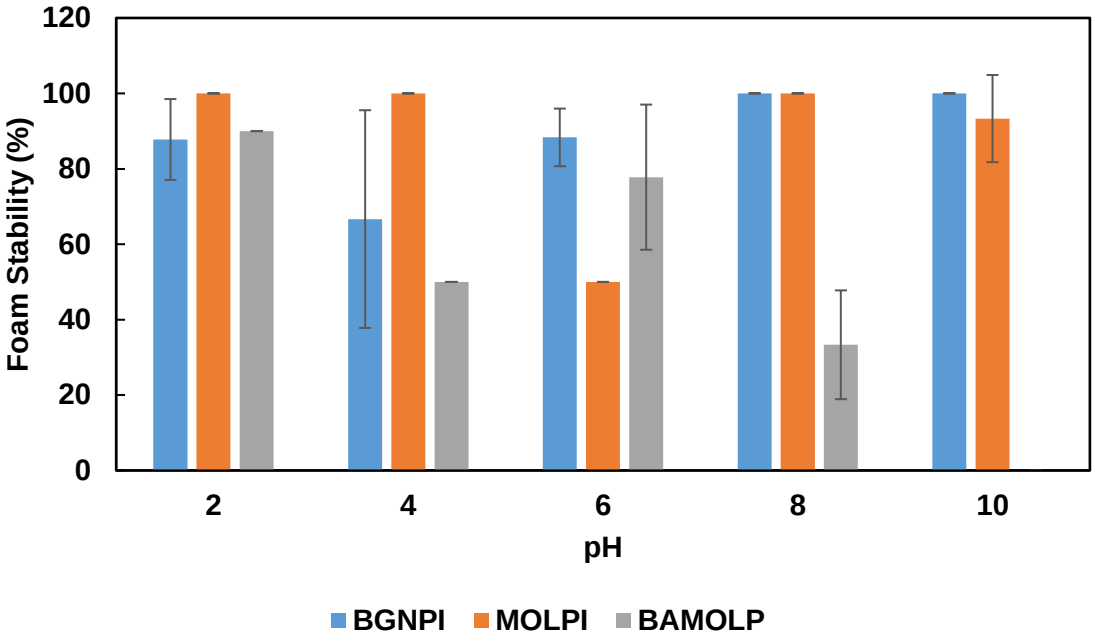

Figure 4.8 Foam stability (%) of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* protein isolate (MOLPI), and BGN and Moringa protein complex (BAMOLP). Error bars are the standard deviation of the mean.

5 *Protein solubility.*

Effects of pH on the protein solubility of BGNPI, MOLPI, and BAMOLP are shown in Figure 4.9. The solubility of BGNPI and BAMOLP showed U-shaped curves in the pH range 2-11, but MOLPI did not show a U-shaped curve. The U-shaped curve shown for solubility of BGNPI and BAMOLP can be linked to the significant ($p < 0.05$) differences in their solubility at the various pH (Table 4.5), the solubility was high at both acidic and alkaline pH and low at pH 4-5. Whereas for MOLPI, there was no significant difference in solubility at the various pH (Table 4.5), hence, a U-shaped curve was not established. The lowest solubility for BGNPI and BAMOLP was at pH 4-5, while the lowest for MOLPI was at pH 2-4. The BGNPI and BAMOLP were soluble in the low acidic pH range and the alkaline pH range. Kudre *et al* (2013) and Adebowale *et al* (2011) reported similar results for BGNPI. Protein molecules are charged at pH values far from the isoelectric point (pI), thus causing repulsion among proteins, which enhances increased solubility. Whereas net charges of protein give zero when the pH is close to isoelectric point (pI) (Kaur and Singh, 2007; Kudre *et al.*, 2013). The protein solubility of BAMOLP compares closely to BGNPI (Figure 4.9, Table 4.5, and Table 4.6). MOLPI showed the lowest solubility at pH 2-4, increased solubility was observed around pH 5-10, similar findings were reported by Ahmed (2016) for *Moringa oleifera* leaf protein concentrate.

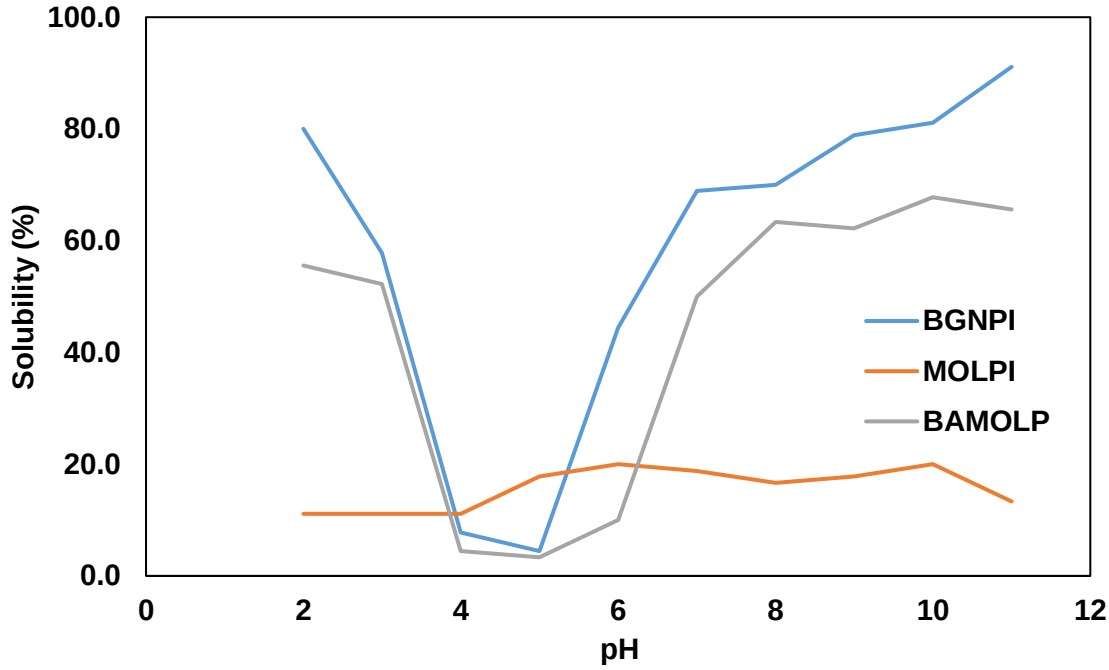

Figure 4.9 Effects of different pH on solubility of protein isolates and complex. BGNPI, Bambara groundnut protein isolate; MOLPI, *Moringa oleifera* leaf protein isolate; BAMOLP, Bambara groundnut-*Moringa oleifera* leaf protein isolate complex

Differences and similarities among the protein isolates and complex are shown in Table 4.6, there was a significant ($p < 0.05$) difference in the solubility at pH 2, 6, 7, 9, 10, and 11 with BGNPI having the highest solubility, followed by BAMOLP and then MOLPI. The observed difference in solubility of BNGPI, MOLPI, and their complex (BAMOLP) could be due to differences in protein type, amino acid composition, degree of association, or dissociation of protein molecules (Table 4.2). At pH 3, 5, and 8 there was no significant ($p > 0.05$) difference in solubility of BGNPI and BAMOLP but the two differ significantly ($p < 0.05$) when compare to the solubility of MOLPI. The observed similarities in the solubility of BGNPI and BAMOLP are because BAMOLP contains about 80% of BGNPI hence their functional properties are expected to be similar. Nevertheless, there was no significant difference at pH 4 in the solubility of BGNPI, MOLPI, and BAMOLP. The solubility of protein at different pH values is an indication of the efficacy of protein isolates incorporated in food systems.

Table 4.5 Variation of protein solubility of BGNPI, MOLPI, and BAMOLP with pH[1]

pH	Protein isolates		
	BGNPI	**MOLPI**	**BAMOLP**
2	80.00 ± 3.34[a]	11.11 ± 3.85[a]	55.56 ± 1.93[a]
3	57.78 ± 1.92[d]	11.11 ± 3.85[a]	52.22 ± 5.09[a]
4	7.78 ± 5.09[f]	11.11 ± 3.85[a]	4.44 ± 1.93[d]
5	4.44 ± 1.93[f]	17.78 ± 3.85[a]	3.33 ± 0.00[d]
6	44.45 ± 3.85[e]	20.00 ± 0.00[a]	10.00 ± 3.33[c]
7	68.89 ± 8.39[c]	18.78 ± 3.65[a]	50. 00 ± 3.33[a]
8	70.00 ± 10.00[c]	16.67 ± 11.55[a]	63.33 ± 3.34[b]
9	78.89 ± 5.09[a]	17.78 ± 1.92[a]	62.22 ± 1.92[b]
10	81.11 ± 1.92[a]	20.00 ± 6.67[a]	67.78 ± 5.09[b]
11	91.11 ± 1.92[b]	13.33 ± 3.34[a]	65.56 ± 1.93[b]

[1]Bambara groundnut (BGN) protein isolate; MOLPI: *Moringa oleifera* leaf protein isolate; BGNPI: BAMOLP: BGN and *Moringa oleifera* leaf protein complex

[2]Mean values of triplicate determinations ± standard deviation. Means within the same column with different superscripts differ significantly ($p < 0.05$)

Table 4.6 Variation of protein solubility among protein isolate as affected by pH

pH	Protein isolates		
	BGNPI	MOLPI	BAMOLP
2	80.00 ± 3.34[a]	11.11 ± 3.85[c]	55.56 ± 1.93[b]
3	57.78 ± 1.92[a]	11.11 ± 3.85[b]	52.22 ± 5.09[a]
4	7.78 ± 5.09[a]	11.11 ± 3.85[a]	4.44 ± 1.93[a]
5	4.44 ± 1.93[a]	17.78 ± 3.85[b]	3.33 ± 0.00[a]
6	44.45 ± 3.85[a]	20.00 ± 0.00[b]	10.00 ± 3.33[c]
7	68.89 ± 8.39[a]	18.78 ± 3.65[c]	50. 00 ± 3.33[b]
8	70.00 ± 10.00[a]	16.67 ± 11.55[b]	63.33 ± 3.34[a]
9	78.89 ± 5.09[a]	17.78 ± 1.92[c]	62.22 ± 1.92[b]
10	81.11 ± 1.92[a]	20.00 ± 6.67[c]	67.78 ± 5.09[b]
11	91.11 ± 1.92[a]	13.33 ± 3.34[c]	65.56 ± 1.93[b]

[1]Bambara groundnut (BGN) protein isolate; MOLPI: *Moringa oleifera* leaf protein isolate; BGNPI: BAMOLP: BGN and *Moringa oleifera* leaf protein complex.

[2]Mean values of triplicate determinations ± standard deviation. Means within the same row with different superscripts differ significantly ($p < 0.05$)

4.7.5 Rheological properties of Bambara groundnut protein isolates (BGNPI), *Moringa oleifera* leaf protein isolates (MOLPI), and BGN and*d Moringa* protein complex, and BGN and *Moringa* protein complex

The viscosity of BGNPI, MOLPI, and BAMOLP are described in the rheogram presented in Figure 4.10. The highest viscosity of BGNPI, MOLPI, and BAMOLP were 9.33, 8.78, and 18, 45 Pas^{-1}, respectively. The BGNPI, MOLPI, and BAMOLP showed a shear-thinning behaviour by decreasing viscosity with increasing shear rate. This behaviour indicates that BGNPI, MOLPI, and BAMOLP are non-newtonian systems with pseudoplastic properties. The viscosity of BGNPI, MOLPI, and BAMOLP against shear stress is presented in Figure 4.11. An increase in shear stress resulted in a decrease in viscosity, which further shows that the protein isolates and the complex display shear thinning (flow more readily as it is stirred) behaviour. A similar result was obtained for pea protein isolates and BGN starch (Taherian *et al.*, 2011; Oyeyinka *et al.*, 2015). Figure 4.12 shows the steady shear flow curve of the protein isolates which further establishes that BGNPI, MOLPI, and BAMOLP are non-newtonian.

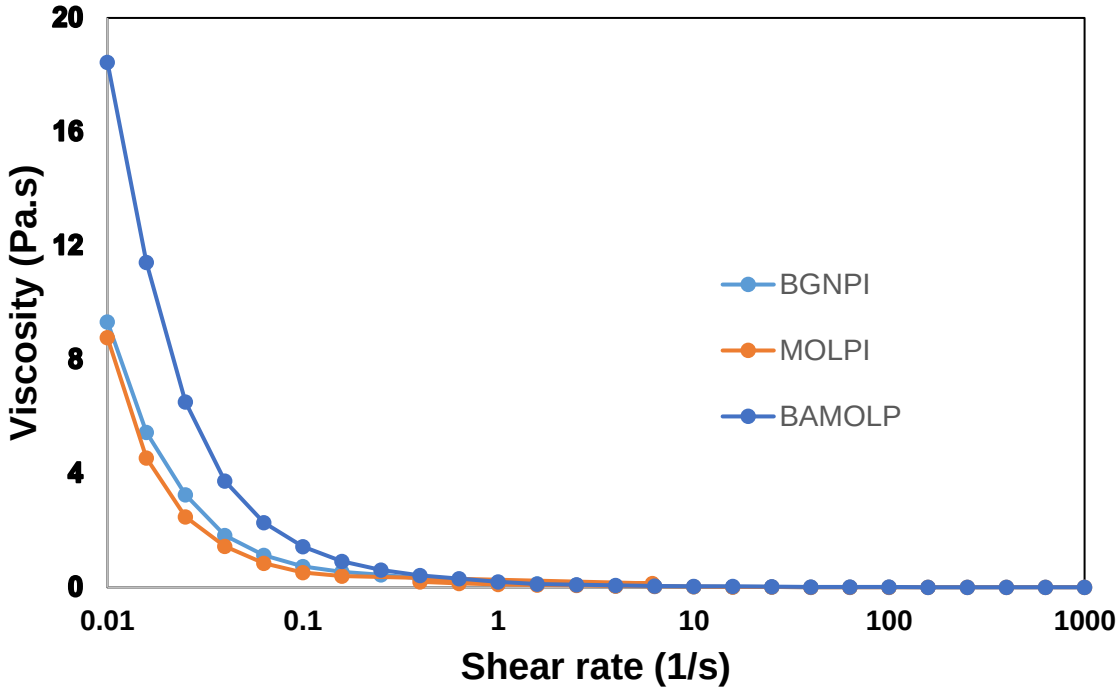

Figure 4.10 Apparent viscosity of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* protein isolate (MOLPI), and Bambara groundnut and *Moringa oleifera* leaf protein complex (BAMOLP) (shear rate as a function)

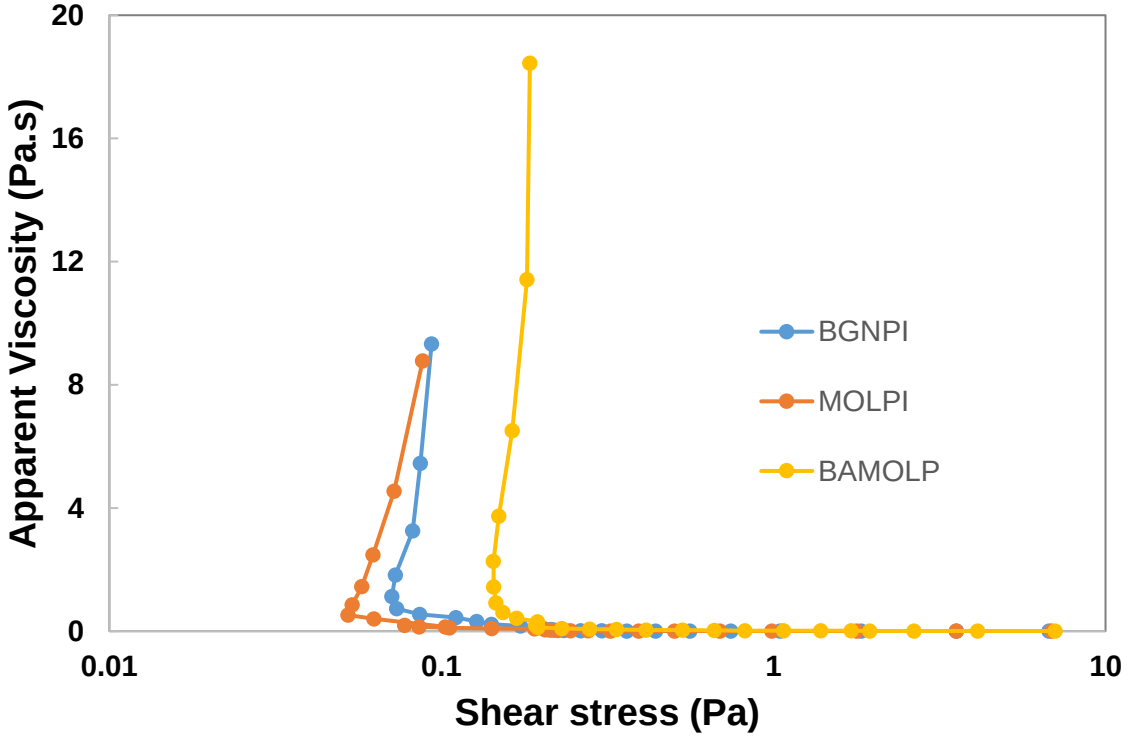

Figure 4.11 Apparent viscosity of Bambara groundnut protein isolate (BGNPI), *Moringa oleifera* protein isolate (MOLPI), and Bambara groundnut and *Moringa oleifera* leaf protein complex (BAMOLP) (shear stress as a function)

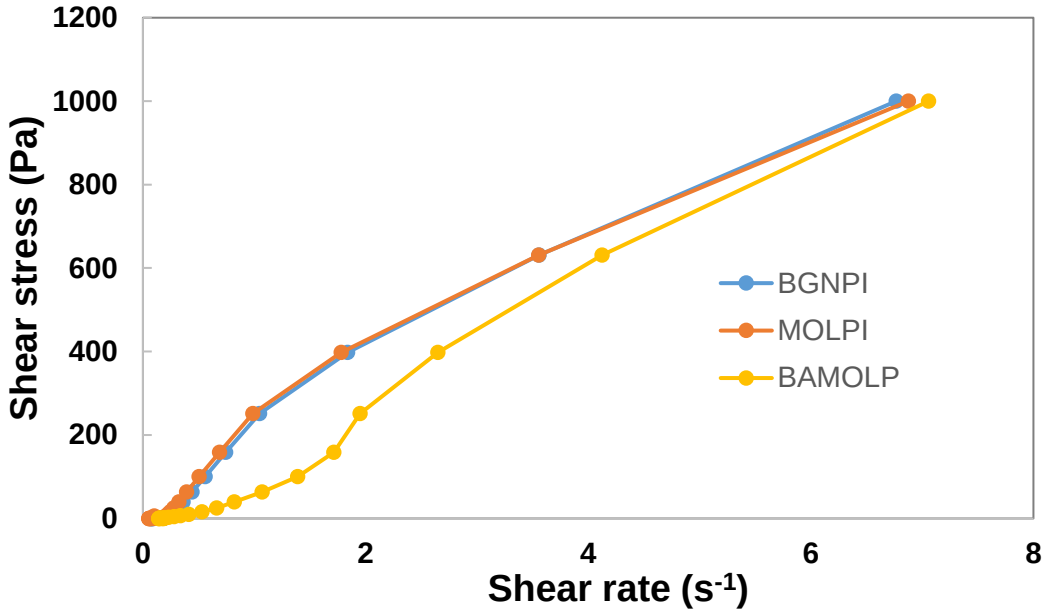

Figure 4.12 Flow Behaviour of Bambara groundnut protein isolates (BGNPI), *Moringa oleifera* leaf protein isolates (MOLPI), and BGN and Moringa protein

4.8 Conclusion

Bambara groundnut and *Moringa oleifera* leaf protein complex BAMOLP was successfully produced from BGNPI and MOLPI. The fat, ash, and carbohydrate content of MOLPI were significantly ($p \leq 0.05$) higher than that of BGNPI and BAMOLP. The protein content of BAMOLP was significantly higher when compared to BGNPI and MOLPI. BAMOLP is a good source of protein and is rich in essential amino acids. It is especially higher in methionine, phenylalanine, and histidine compared to whey, pea, brown rice, soy, hemp, and wheat protein and can therefore be used as an alternative in applications where those proteins are desired. Complementing *Moringa oleifera* protein with BGN protein was beneficial as the methionine in *Moringa oleifera* was higher than BGN protein, hence the complex (BAMOLP) was higher in methionine. The amino acid content of BAMOLP was higher in threonine, phenylalanine lysine, and leucine when compared to FAO/WHO reference pattern. Due to the appreciable content of lysine in BAMOLP, it can be used as a supplementary protein to cereals, which are deficient

in lysine. BAMOLP is, therefore, a potential functional food ingredient and sustainable raw material for the food industry.

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CHAPTER FIVE

PHYSIOCHEMICAL AND NUTRITIONAL CHARACTERISTICS OF READY-TO-USE THERAPEUTIC FOOD PREPARED USING BAMBARA GROUNDNUT-*MORINGA OLEIFERA* LEAF PROTEIN COMPLEX

ABSTRACT

The utilisation of local raw material in the production of ready-to-use therapeutic food (RUTF) is worthy of exploration for the replacement of full-fat milk, peanut butter, mineral, and vitamin mix used in the formulation. The objective of this study was to produce snack bars that will meet the protein requirement set by World Health Organisation (WHO) for RUTF (13-16% by weight) using the Bambara groundnut-*Moringa oleifera* leaf protein complex and profile its nutritional properties. The BAMOnut snack bars were simulated using the mixture preparation procedure in Superpro Designer to determine Bambara groundnut-*Moringa oleifera* leaf protein complex, *Moringa oleifera* leaf powder, egusi, oats, and millet in different proportions. Three bars formulated were; BAMOnut-OB3 (BAMOnut Bar enriched with oats and 3% BAMOLP, BAMOnut-MB2 (BAMOnut Bar enriched with millets and 2% BAMOLP), and BAMOnut-OMB5 (BAMOnut Bar enriched with oats, millets, and 5% BAMOLP). The BAMOnut snack bars were assessed for physical characteristics (morphology, water activity, colour, texture profile analysis), nutritional composition (amino acid and mineral profile), proximate composition and bench-top sensory properties. The physical appearance of BAMOnut-OB3 (snack bar enriched with oats and 3% BAMOLP) was firmer and less crumbling but had a larger particle size. Eleven mineral components were in the snack bars and higher compared to RUTF except for sodium content in BAMOnut-OB3. BAMOnut-OB3 had the least water activity, lightest colour with the best amino acid profile. There was no significant difference in the texture of the BAMOnut snack bars. The formulation with a higher concentration of MOLP has a lower sensory rating in appearance, colour, aroma, taste, and overall acceptability. Moisture (4.9%), protein (14.1%), fat (19.3%), CHO (59.7%), and energy (468.6 Kcal/100g), of BAMOnut-OB3, compares favourably with the requirement for RUTF (2.5, 13-16, 26-36, 41-58% for moisture, protein, carbohydrate, and 520-550 Kcal/100g for energy), respectively. Two principal components (PC) were identified, PC 1 (78.8%), and PC2 (21.2%) which accounted for 100% of the variance in the physical and chemical composition of the BAMOnut snack bars. Principal component one correlates with BAMOnut-OB3 which is high in unsaturated fatty acids, has a strong texture, redder, yellower and lighter in colour and is also high in protein. Principal Component 2, which is 21.2% of the variability, is associated with BAMOnut-MB2, which is high in ash content, moisture, and protein. Local raw materials can be successfully used in the production of an alternative RUTF for severely acutely malnourished children and as well-nutritious snacks for adults.

5.1 Introduction

Bambara groundnut-*Moringa oleifera* leaf protein complex (BAMOLP) is an innovative product that was produced in Chapter 3. BAMOLP is a combination of Bambara groundnut protein isolate and *Moringa oleifera* leaf protein isolate in ratio 9:2, respectively. BAMOLP is a potential functional food ingredient and sustainable raw material for the food industry due to its nutritional profile as established in Chapter 4. BAMOLP is a good source of protein and is rich in essential amino acid. It is higher in methionine compared to that in whey, pea, and soy protein as reported by Stone *et al*. (2015). It is also higher in phenylalanine compared to that in whey, pea, brown rice, soy, hemp, and wheat protein as reported by Malomo *et al*. (2014) and Stone *et al*. (2015), and can therefore be used as an alternative in applications where those proteins are required. The amino acid content of BAMOLP was higher in threonine, phenylalanine lysine, and leucine when compared to FAO/WHO reference pattern (Adam *et al.*, 2015). Due to the appreciable content of lysine in BAMOLP, it can be used as a supplementary protein to cereals, which are deficient in lysine.

Ready-to-use therapeutic foods (RUTF) have been confirmed operative in the management of severe acute malnutrition, but some ingredients needed for its production are not available locally. Plumpy'Nut, Imunut, and BP-100 are the commercial forms of RUTF. Both BGN and MOLP are nutrient-dense foods with quality protein that can be used as local raw material in the production of RUTF. They are both rich in amino acids, minerals, and vitamins. Nevertheless, not much application of their food value is known especially in malnutrition. Complementing the protein of BGN with MOLP will provide a complete protein that will compete with other ingredients used in the production of RUTF, and will meet the protein requirement (10-12% of the total energy) for RUTF set by WHO.

Ingredients for standard RUTF are full-fat milk, peanut, vegetable oil, sugar, and mineral and vitamin mix, while the commonly used ingredients for the alternative RUTF are a legume (almond, groundnut, lentil, and soybean), cereal/grain (maize and oats), milk (whey protein concentrate), oil (canola, palm, sunflower, soybean, and coconut), sugar and cocoa powder. This study aimed at the development of a snack bar that will meet RUTF requirement through fortification with Bambara groundnut-*Moringa oleifera* leaf protein complex (BAMOLP). The BAMOLP will increase protein content and a serves as a replacement for the full milk powder, *Moringa oleifera* leaf powder (MOLP) for the vitamin and mineral mix, egusi melon (*Citrullus lanatus sub-Mucosospermus*) as a replacement for peanut which is often exposed to aflatoxin, millet and oat as energy source (Manary, 2006; Weber *et al.*, 2017).

5.2 Materials and Method

5.2.1 Sources of materials and equipment

The BGN seeds were purchased from Triotrade Johannesburg, South Africa, MO leaf from *Moringa* Africa, Johannesburg, millets from AGT Foods Africa, Cape Town, and egusi from a local seed store in Cape Town. Chemicals were purchased from Merck Pty Ltd, South Africa. Food ingredients were purchased from Bidvest FoodService, Cape Town. The equipment was obtained from the Department of Food Science and Technology, Cape Peninsula University of Technology, Cape Town, South Africa. Figure 5.1 outlines the processes and analyses that were carried out in this chapter.

5.2.2 Pre-processing of millet and melon seed (Egusi)

The millet was dehulled by boiling, and treatment with calcium hydroxide to remove the seed coat. A 5 g calcium hydroxide was added to 1 L boiling water, after which 1 kg millet was added to the solution. The millet in the solution was stirred with a spoon. Thereafter drained, and poured into 1% citric acid. It was again, rinsed, washed, drained, and dried at 50^{o}C for 4 hours, it was then crushed and ground in a mortar and pestle to reduce the particle size.

The egusi was sorted to remove dirt, after which it was washed, drained, and dried in the dehydrator at 50^{o}C for 6 h and roasted at 180^{o}C for 6-7minutes. It was afterwards milled in a Kenwood blender at setting 3, and packed in a zip-locked bag, and kept in the fridge at 4^{o}C until further use.

5.2.3 Simulation of ingredient proportions to meet the Ready-to-use therapeutic requirement using Superpro designer.

The proximate compositions of BAMOLP, egusi, MOLP, and oats used in the simulation are outlined in Table 5.1. The cereals for the bars were millets and oats used as the source of energy. The oilseed chosen for the base to replace the commonly used peanut butter was egusi. It was desired to produce three bars consisting of BAMOLP + MOLP + oats + egusi, BAMOLP +MOLP + millet + egusi, and BAMOLP + MOLP + millet + oats +egusi. The objective in each case was to obtain bars with fat: 45 – 60% total energy or 26 – 36% by weight, protein: 10 – 12% total energy or 13 – 16% by weight, and moisture 2.5% max.

The mixture preparation procedure in SuperPro designer (Version 9) was used to obtain the appropriate proportion of components to meet the optimisation goal. The mixture preparation procedure simulates an intelligent mixer that automatically adjusts the flow of its input streams to meet the objective goal. The objective goal for each bar is detailed in Table 5.2. The simulation results are depicted in Figure 5. 2.

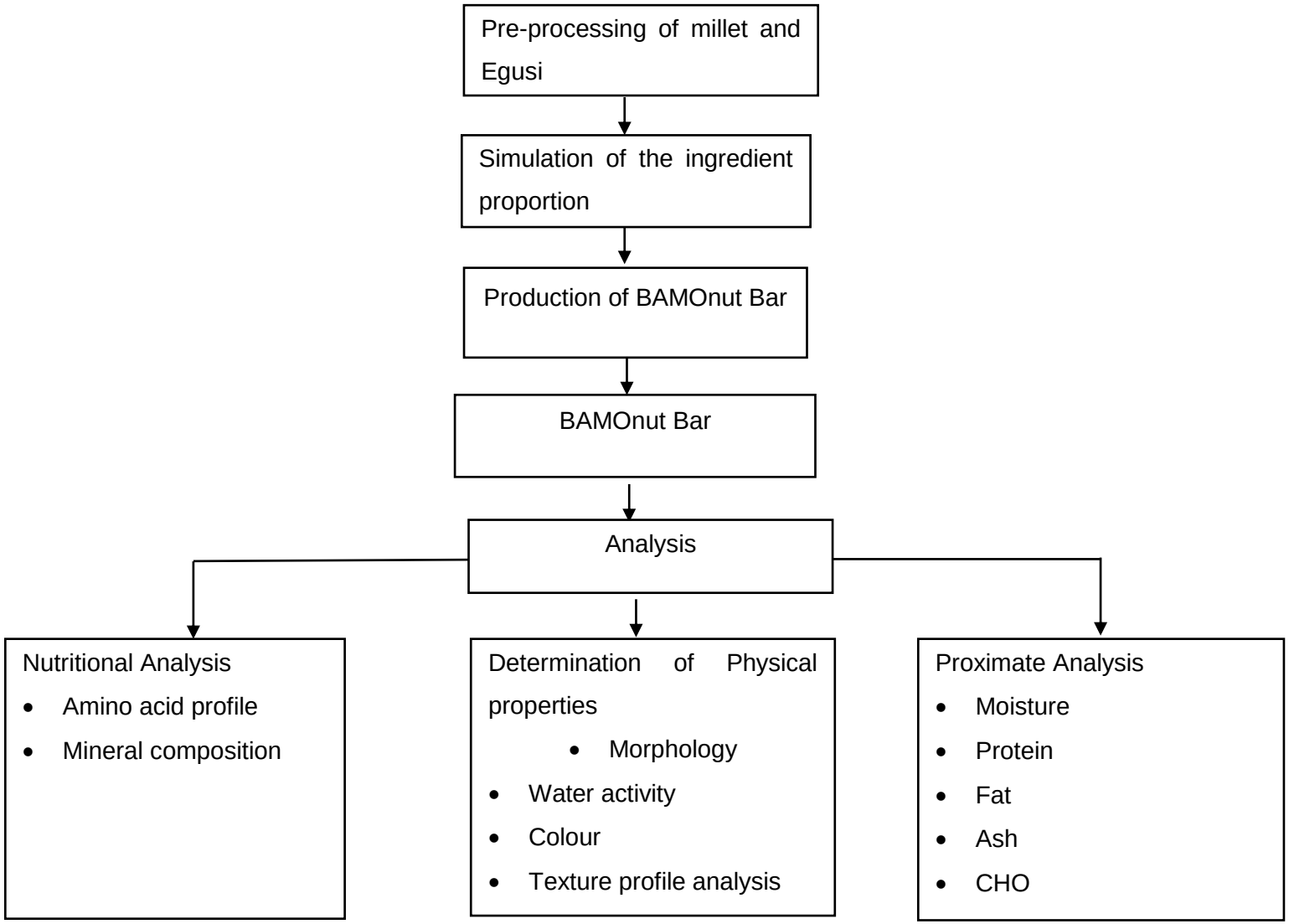

Figure 5.1 Chapter 5 outline

Table 5.1 Proximate composition of ingredients

	Fat	Protein	Water	Others	References
BAMOLP	8.07	63.51	2.42	26.0	Chapter 3, section 3.7.8
Egusi	49.0	28.0	5.10	17.9	USDA (2018)
MOLP	6.0	33.3	5.6	55.1	Busani *et al* (2011)
Oats	6.52	13.15	10.84	69.49	USDA (2018)
Millets	3.33	10.00	4.00	82.67	Badau *et al*., (2002)

BAMOLP: Bambara groundnut-*Moringa oleifera* protein complex, MOLP *Moringa oleifera* leaf powder.

Table 5.2 Optimisation goal for Bambara groundnut and *Moringa oleifera* snack bar

Chemical composition (%)	**Oats with 3% BAMOLP**	**Millet with *2%* BAMOLP**	**Oats, millet with *5%* BAMOLP**
Fat	26	26	24.9
Protein	24	27	22.6
Water	7.29	5	5.9

BAMOLP: Bambara groundnut-*Moringa oleifera* protein complex.

5.2.4 Production of Bambara groundnut and *Moringa oleifera* snack bar

Three varieties of Bambara groundnut and *Moringa oleifera* (BAMOnut) snack bar were produced. The main constituents of the bars were from the simulation as listed in Table 5.3, other ingredients namely, 2 g cocoa powder, 30 g icing sugar, 15 g canola oil, and 45 g honey were added to each batch. First, the dry ingredients were weighed into the Kenwood blender, and mixed on speed setting 1 for 2 min, and speed setting 3 for 3 minutes. It was thereafter transferred into a mixing bowl, wet ingredients were added, and mixed until well combined. A 250 g batch was produced, the mixture was taken out of the bowl, and placed in a pre-lined baking pan. The mixture was baked in a pre-heated oven at 160^{o}C for 30 min and cooled at room temperature, thereafter it was cut into a rectangular shape.

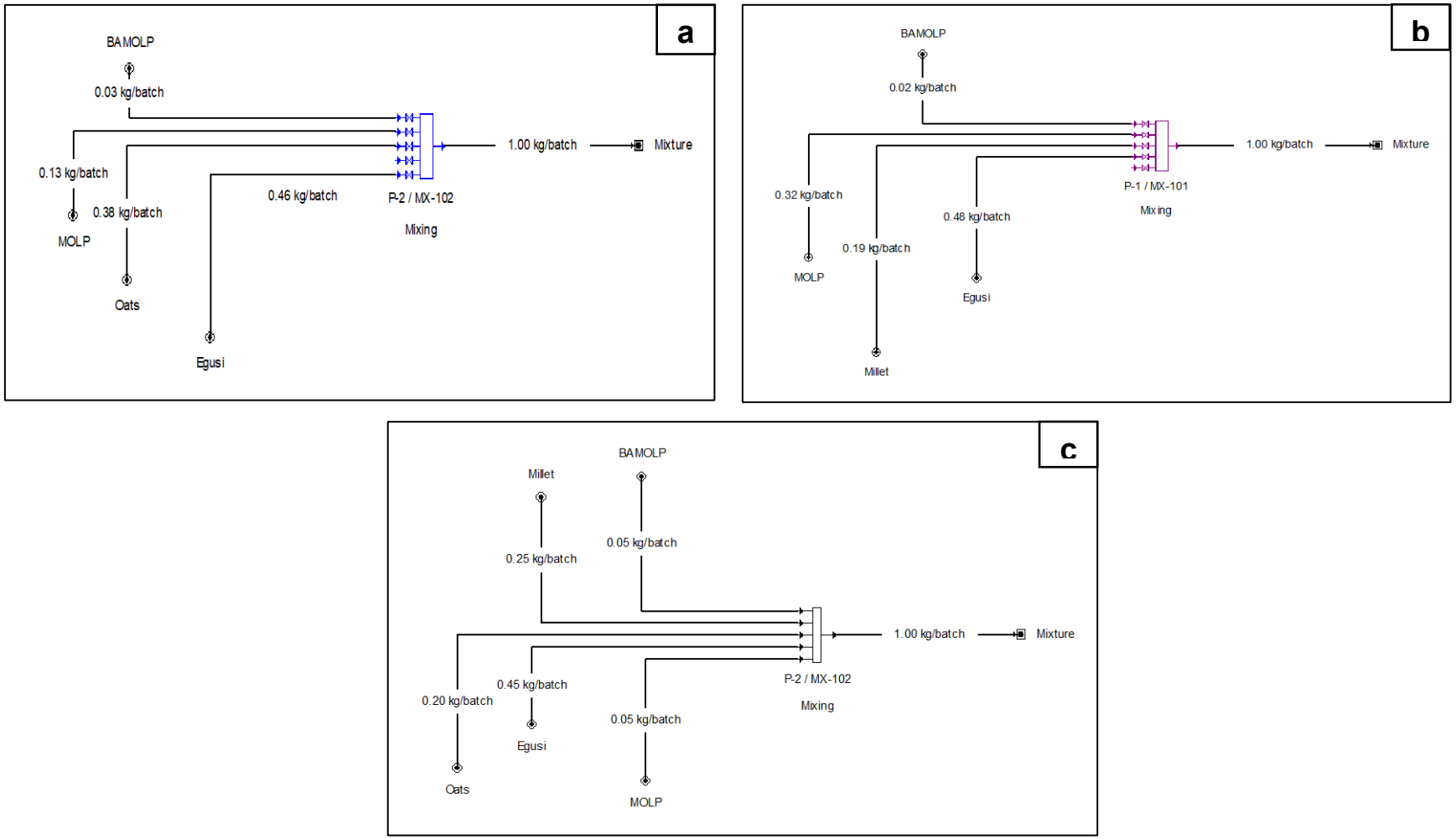

Figure 5.2 Process flow diagram of Bambara groundnut-*Moringa oleifera* snack bar enriched with BAMOLP at low, medium, and high concentrations, a: oats plus 3% BAMOLP, b: millets plus 2% BAMOLP, c: oats, millets, and 5% BAMOLP

Table 5.3 Formulation of three varieties of BAMOnut-Bar.

Ingredients (%)	OB3	MB2	OMB5
BAMOLP	3.0	2.0	5.0
Egusi	46.0	48.0	45.0
MOLP	13.0	32.0	5.0
Oats	38.0.0	-	20.0
Millet	-	19.0	25.0

BAMOnut: Bambara groundnut-*Moringa oleifera* snack bar, OB3: BAMOnut snack bar enriched with oats, and *3%* BAMOLP, MB2: BAMOnut snack bar enriched with millet and 2% BAMOLP, OMB5: BAMOnut snack bar enriched with oats, millet, and 5% BAMOLP. BAMOLP: Bambara groundnut-*Moringa oleifera* protein complex, MOLP: *Moringa oleifera* leaf powder.

5.3 Physical properties of BAMOnut

5.3.1 Morphology (characterisation) of BAMOnut bars

1 Scanning electron microscopy-energy dispersive X-ray spectrometry (SEM-EDX) analysis of BAMOnut snack bars.

Scanning electron microscope (SEM) analysis was carried out using Zeiss MERLIN (Carl Zeiss Microscopy, Germany). Samples were placed on carbon double-sided adhesive tape that was mounted on Aluminum SEM stubs. They were observed with a scanning electron microscope, Zeiss MERLIN (Carl Zeiss Microscopy, Germany) operated at an acceleration voltage of 20 kV.

2 Scanning transmission electron microscopy (STEM)

Basic STEM preparation was done for the BAMOnut snack bar samples. Pieces of the samples were dispersed in Ethanol, thereafter sonicated for an hour. A drop of the solution was placed in a Petri dish for each sample and a FORMVAR coated 200 Mesh Cu TEM grid was placed in the droplet. The Grid was then rinsed in a droplet of distilled water and placed in a droplet of UA zero (uranium free urinal acetate).

5.3.2 The water activity of BAMOnut Bars

Water activity (a_w) of BAMOnut Bar (oat with Moringa, millet with *Moringa oleifera leaf powder,* and oat and millet with Moringa) was measured using the Novasina Ms 1 Set a_w meter, which uses a cell protection filter. The measurement cell was calibrated with salt humidity standards of 53, 75, and 90%. The three variations of BAMOnut were transferred individually into the sample container. The sample container was placed inside the Novasina analyser and the cell

measuring protection filter was immediately closed. The water activity reading was observed for stability and the values were recorded (Novasina General Catalogue, 2012). The test was carried out in triplicate.

5.3.3 Colour measurement

The colour of the BAMOnut snack bars was measured using a Spectrophotometer (Model CM-5 45º/0º standard, Konica Minolta Sensing, Osaka, Japan), set at standard observe 10º and D65. The instrument was calibrated using a black tile (L* = 5.49, a* = 7.08, b* = 4.66) and a white tile (L* = 93.41, a* = -1.18, b* = 0.75), followed by zero calibration. The L* coordinate is lightness, 100 represents white and closer to 0 represents black, a* (chromaticity coordinate +a* = red and –a* = green), b* (chromaticity coordinate +b* = yellow and –b* = blue), C* = Chroma, h = hue angle (0º = +a*, 90º = +b*, 180º = -a* and 270º = -b*).The BAMOnut bars were crushed using a mortar and pestle. The samples were placed in a sample-dish (30 mm diameter) and reflectance was measured for L*a*b* and L*C*h* colour space system. Measurements were taken in triplicates for each sample (one reading = average of three readings per rotated position). The total colour difference (ΔE) was estimated using equation 5.1.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{Equation 5.1.}$$

5.3.4 Textural properties of BAMOnut Bar

BAMOnut Bar was analysed for hardness, springiness, cohesiveness, chewiness, using the Instron Model 3322. For the texture profile analysis (TPA), the BAMOnut bar was cut into 4 cm length x 1 cm height with a knife and put on the support plate and then compressed with compression anvil with pre-test condition set at 2.00 mm/s, test at 2.0 mm/s, and post-test at 2.00 mm/s. The BAMOnut bar was cut into 2.5 cm length by 1 cm height for the puncture test and the pre-test speed was set at 1 mm/s, the probe used was 5 mm in diameter. The cutting test was performed using the Warner-Bratzler Shear Force (WBSF) method. The WBSF blade was mounted to the Instron device, the crosshead speed for the pre-test was 200 mm/min and 250 mm/min for the test. The BAMOnut snack bar for the test was cut into 2.5 cm length x 1 height cm for the test. For the texture profile analysis (TPA), BAMOnut bar (4 cm length x 1 cm height) was cut with a knife and put on the support plate and then compressed with compression anvil with pre-test condition set at 2.00 mm/s, test at 2.0 mm/s, and post-test at 2.00 mm/s.

5.4 Nutritional and proximate composition of BAMOnut Bar

5.4.1 Amino acid

The amino acid in the BAMOnut snack bar was determined using the Waters Altra performance liquid chromatography (UPLC) separation with UV detection after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC).

5.4.2 Proximates

Protein determination was done using using Truspec Nitrogen Analyser (LECO) method, total fat according to AOAC (2005) method 996.06, moisture and ash according to AOAC (2005) method 934.01 and 923.03, respectively. The carbohydrate was determined by difference. Energy value was calculated using conversion factors of 4, 4, and 9 Kcal g^{-1} for protein, carbohydrate, and lipids, respectively.

5.5 Sensory

A benchtop sensory evaluation was conducted using 10 panellists consisting of students and staff of the Cape Peninsula University of Technology were served 3 samples of BAMOnut Bar. The bars were rectangular and range from 58.99 to 48.04 g in size. The BAMOnut bars were produced 4 h before the sensory. The samples were presented in a zip-locked bag with three-coded digits. A cup of water was provided to reset the palate in-between the tastings. A score sheet that consisted of three-coded samples with a 5-point hedonic scale ranging from 1 = dislike extremely to 5 = like extremely was given to each panellist. They were instructed to rate each sample individually on its merit on a five-point hedonic scale rating for appearance, colour, taste, aroma, texture, and overall acceptability.

5.6 Statistical analysis

All experiments were conducted in triplicate. To determine mean differences between treatments, obtained data were subjected to multivariate analysis of variance (ANOVA). Where differences existed, separation of means was carried out using Duncan's multiple range test (IBM SPSS, version 25). Application of Kruskal Wallis test (non-parametric ANOVA) where normality test was violated.

5.7 Result and Discussion

5.7.1 Physical characteristics of BAMOnut Bar.

The physical appearance, colour, microstructure, and water activity attributes of the BAMOnut-snack bars are described in this section.

1 *Physical appearance*

BAMOnut snack bars are presented in Figure 5.3. BAMOnut-bar enriched with oats and 3% BAMOLP (BAMOnut-OB3) shown in Figure 5.3a appeared more attractive and firmer than BAMOnut-bar enriched with millet and 2% BAMOLP (BAMOnut-MB2) and BAMOnut-bar enriched with oats, millet, and 5% BAMOLP (BAMOnut-OMB5). The difference could be due to the absence of water in BAMOnut-OB3, whereas, cooked and wet millet was incorporated in the production of BAMOnut-MB2 and BAMOnut-OMB5. The wetness of the cooked millets may have contributed to reduced firmness in both BAMOnut-MB2 and BAMOnut-OMB5.

Figure 5.3 Physical appearance of BAMOnut snack bars- a: BAMOnut enriched with oats and 3% BAMOLP (BAMOnut-OB3), b: BAMOnut enriched with millet and 2% BAMOLP (BAMOnut-MB2), and c: BAMOnut enriched with oats, millet, and 5% BAMOLP (BAMOnut-OMB5).

2 *Colour attributes*

The colour of BAMOnut snack bars is shown in Table 5.4. The average lightness for BAMOnut-OB3 (BAMOnut snack bar enriched with oats, and 3% BAMOLP), BAMOnut-MB2 (BAMOnut snack bar enriched with millets, and 2% BAMOLP), and BAMOnut-OMB5 (BAMOnut snack bar enriched with oats, millets, and 5% BAMOLP) were 25.82, 19.16, and 21.60 respectively. The redness of BAMOnut-OB3, BAMOnut-MB2, and BAMOnut-OMB5 were 7.23, 3.74, and 4.66, respectively. The yellowness of BAMOnut-OB3, BAMOnut-MB2, and BAMOnut-OMB5 were 19.53, 11.08, and 13.08 respectively, and the chroma were 20.83, 11.70, and 14.59, respectively, while the hue angles were 69.67^{o}, 71.24^{o}, and 71.34^{o} respectively. All the bars differed significantly ($p < 0.05$) in lightness, redness, yellowness, and chroma. The hue angle of BAMOnut-snack bars did not differ significantly

Chroma describes the intensity of the colourfulness perceived by humans (Pathare *et al.*, 2013), the BAMOnut snack bars were not vivid, their chroma ranged from 11.70 to 20.83 which is far from the saturation point of 120. Hue is how the colour of an object is perceived.

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Table 5.4 Colour attributes of Bambara groundnut-*Moringa oleifera* snack bar

BAMOnut Bar	Colour parameters				
	L*	a*	b*	C*	h^o
OB3	25.82 ± 0.39[a]	7.23 ± 0.15[a]	19.53 ± 1.15[a]	20.83 ± 1.13[a]	69.67 ± 0.70[a]
MB2	19.16 ± 0.07[c]	3.74 ± 0.20[c]	11.08 ± 1.32[c]	11.70 ± 3.56[c]	71.24 ± 1.19[a]
OMB5	21.60 ± 0.55[b]	4.66 ± 1.95[b]	13.83 ±1.09[b]	14.59 ± 1.03[b]	71.34 ± 1.25[a]

Mean values ± standard deviation of triplicate determination. Mean values in the same column followed by different letters are significantly ($p < 0.05$) different; L*: Lightness; a*: Redness: b*: Yellowness; C*: Chroma, h^o: Hue angle, ΔE: Colour difference. BAMOnut: Bambara groundnut-*Moringa oleifera* snack bar, OB3: BAMOnut snack bar enriched with oats and 3% BAMOLP, MB2: BAMOnut snack bar enriched with millet and 2% BAMOLP, OMB5: BAMOnut snack bar enriched with oats, millet, and *5%* BAMOLP

The hue angle of the bars indicated that BAMOnut bars are dominated by a yellowish colour as they are close to a hue-angle of 90^{o}, which represents pure yellowness, (chroma 11.7-20.83, hue; 69.67-71.34). BAMOnut-OB3 bar produced with oat and 3% BAMOLP had a higher L* .The higher L* is an indication of greater lightness than the other two bars. Nevertheless, all the three bars can be described as dark in colour as they are closer to zero which indicates black on the colour scale (19.2 to 25.8). Hence, the BAMOnut bar can be described as dark yellowish-red not saturated.

The external appearance of the BAMOnut snack bars showed a darker colour (Figure 5.3) which could be a result of Maillard and caramelization reactions between protein, sugar, and honey in the formulation during baking (Khouryieh and Aramouni, 2013). Colour is a key attribute that influences consumer's acceptability of a food product at the time of purchase (Pathare *et al.*, 2013).

The colour difference (ΔE) between BAMOnut-OB3 and BAMOnu-MB2, BAMOnu-MB2 and BAMOnu-OMB5, and BAMOnu-OB3 and BAMOnu-OMB5 were determined to be 27.89, 4.10, and 7.69, respectively. The colour differences are perceivable since a colour difference > 1 is defined as a just noticeable difference, where the observer can notice the difference. The sensory evaluation confirmed that the consumers perceived differences in colour of the BAMOnut snack bars. However, the snack bars were acceptable. Devisetti *et al*. (2016) also produced acceptable ready-to-eat snacks with alkali pre-treated 20% *Moringa* leaf flour. BAMOnut-snack bar produced with oats and 3%, BAMOLP (BAMOnut-OB3) may be preferred because it is the lightest of the three variations

3 *Microstructure of BAMOnut snack bars*

Scanning electron micrographs (SEM) of BAMOnut snack bars are shown in Figure 5.4, the particles are in micrometre scales (40 µm) and agglomerated. The BAMOnut snack bars had particles with irregular shapes and different sizes. BAMOnut-OB3 had larger particles compared to BAMOnut-MB2 and BAMOnut-OMB5. The quantitative analysis using energy dispersion X-ray diffraction (EDX) shown in Figure 5.5 and Table 5.5 indicated that 11 elements were in the BAMOnut snack bars. The EDX spectrum showed the presence of sodium (0.7-9.7%), magnesium (5.4-15.7%), phosphorous (17.3-25.1%), potassium (24.7-30.4%), calcium (5.2-22.8%), iron (1.4-1.8%), nickel (1.2-3.2%), copper (4.1-16.3%), zinc (0.3-5.3%), selenium (0.7-3.1%), iodine (2.7-9.4%). The elements present are expressed in weight percentage and in proportion to the total elements of the area analysed.

The mineral components of the BAMOnut snack bars are not significantly different, except for sodium, where BAMOnut-OMB5 was significantly ($p < 0.05$) higher than that of BAMOnut-OB3 and BAMOnut-MB2 (Table 5.5).

Scanning transmission electron microscopy (STEM) further revealed that the surface of the BAMOnut snack bars, varied significantly as shown in Figure 5.6.

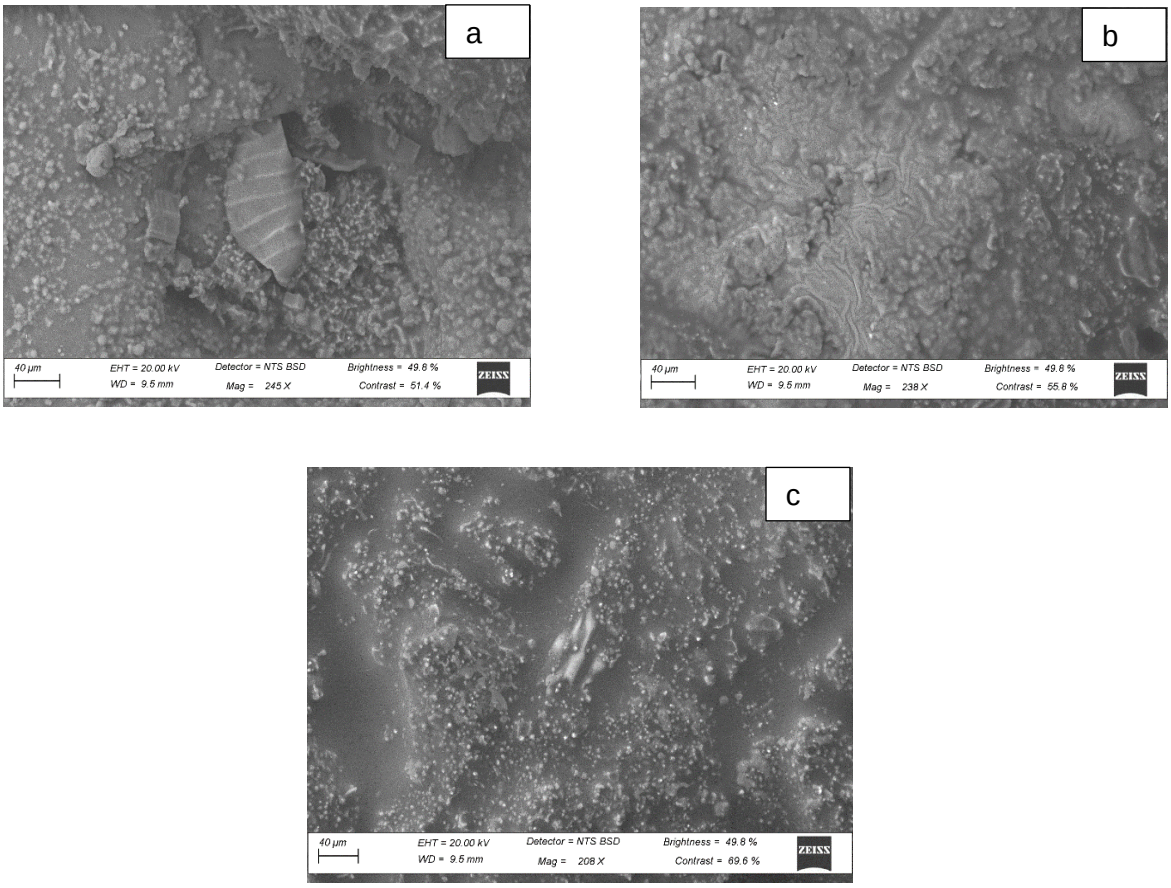

Figure 5.4 Scanning Electron Micrograph of Bambara groundnut-*Moringa oleifera* snack bars (BAMOnut). (a) show morphology of BAMOnut enriched with oats and 3% BAMOLP, (b); BAMOnut enriched with millet and 2% BAMOLP, (c); BAMOnut enriched with oats, millet and 5% BAM BAMOLP, (magnification x245 for a, b: :x238, C: x206). All the micrographs are on a scale of 40 µm.

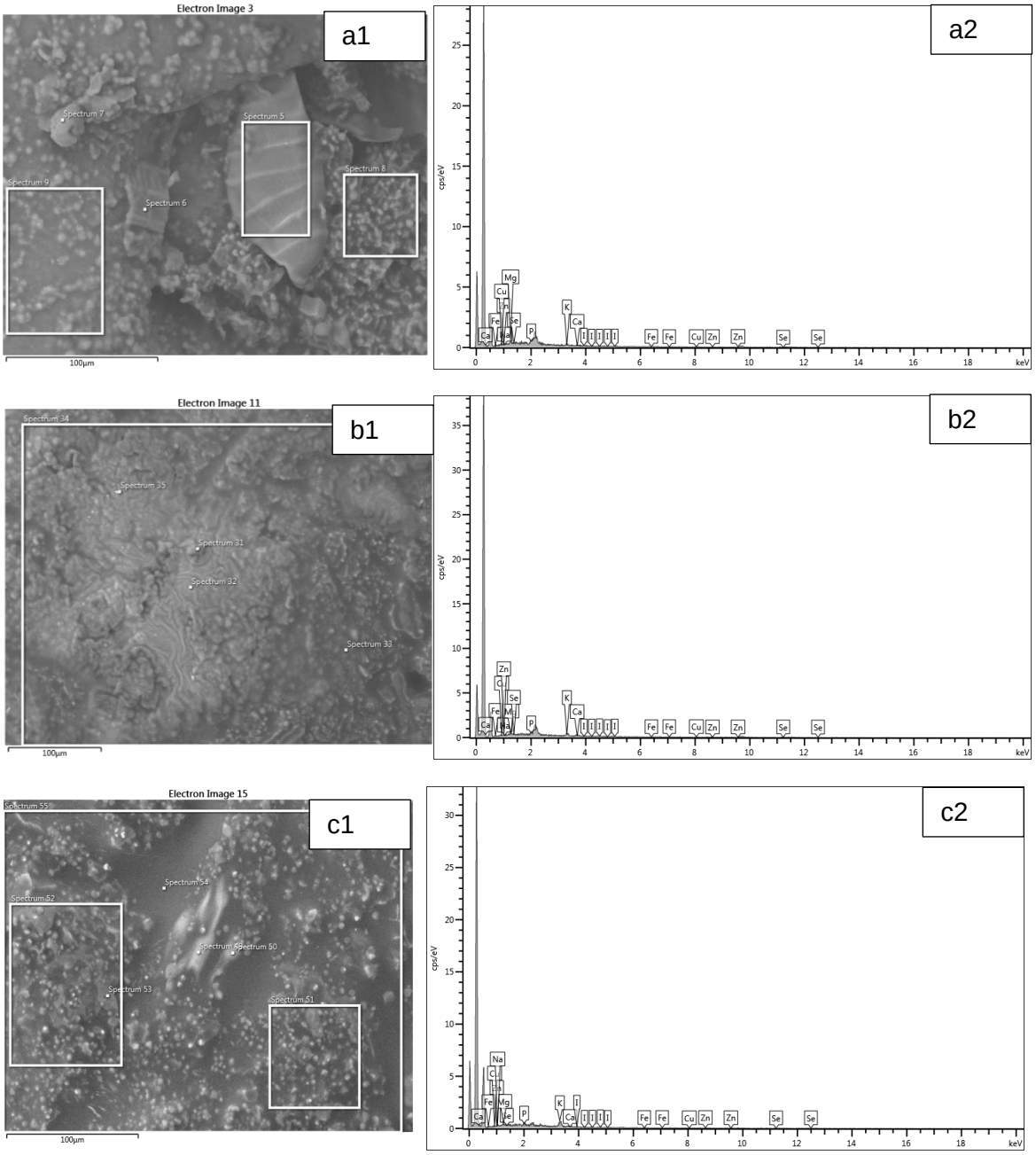

Figure 5.5 EDX characterization spectrum obtained for BAMOnut snack bar; a: of BAMOnut enriched with oats and 3% BAMOLP, b; BAMOnut enriched with millet and 2% BAMOLP, c; BAMOnut enriched with oats, millet and 5% BAMOLP. All the micrographs are on a scale of 100 µm. Visible peaks confirm the presence of sodium, magnesium, phosphorous, potassium, calcium, iron, nickel, copper, zinc, selenium, iodine

Table 5.5 Mineral composition of BAMOnut

	BAMOnut Bar		
Mineral (weight %)	OB3	MB2	OMB5
Sodium	0.0 ± 0.0[a]	0.7 ± 1.2[a]	9.7 ± 3.5[b]
Magnesium	15.7 ± 3.2[a]	13.5 ± 7.4[a]	5.4 ± 6.0[a]
Phosphorous	25.1 ± 4.8[a]	17.3 ± 13.4[a]	18.4 ± 5.2[a]
Potassium	24.7 ± 8.8[a]	29.8 ± 1.7[a]	30.4 ±29.2[a]
Calcium	8.1 ± 2.0[a]	22.8 ± 13.7[a]	5.2 ± 6.1[a]
Iron	1.8 ± 1.6[a]	1.1 ± 1.8[a]	1.3 ± 2.3[a]
Nickel	1.2 ± 2.1[a]	3.2 ± 2.9[a]	2.9 ± 5.0[a]
Copper	9.5 ± 3.8[a]	4.1 ± 4.7[a]	16.3 ± 25.2[a]
Zinc	5.3 ± 9.0[a]	1.8 ± 2.3[a]	0.3 ± 0.5[a]
Selenium	1.1 ± 2.0[a]	3.1 ± 2.3[a]	0.7 ± 1.7[a]
Iodine	7.5 ± 8.4[a]	2.7 ± 0.7[a]	9.4 ± 7.0[a]

BAMOnut: Bambara groundnut-*Moringa oleifera* snack bar, OB3: BAMOnut snack bar enriched with oats and 3% BAMOLP, MB2: BAMOnut snack bar enriched with millet and 2% BAMOLP, OMB5: BAMOnut snack bar enriched with oats, millet, and *5%* BAMOLP, RUTF: Ready-to-use therapeutic food

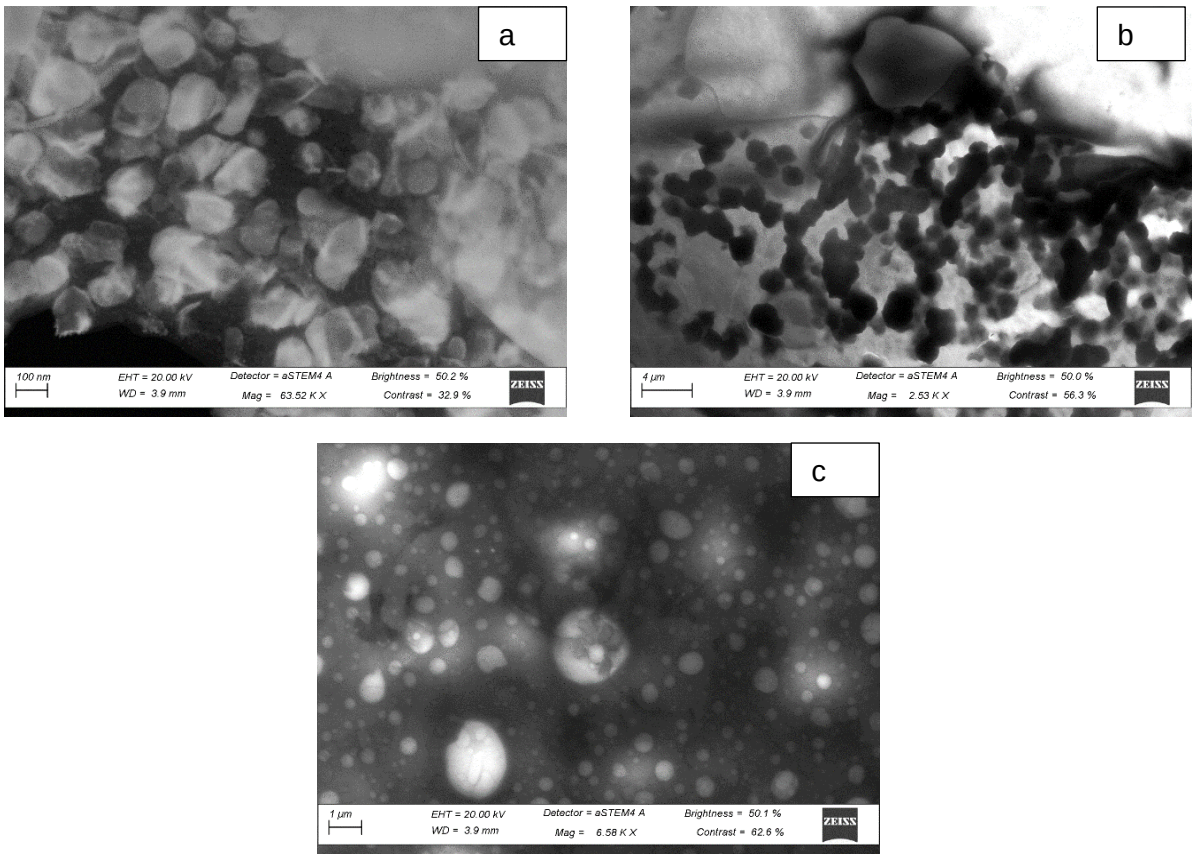

Figure 5.6 Scanning Transmission Electron Micrograph of Bambara groundnut-*Moringa oleifera* bars (BAMOnut). (a) show the morphology of BAMOnut enriched with oats and 3% BAMOLP on 100 nm scale, b; BAMOnut enriched with millet and 2% BAMOLP on 4 µm scale, c; BAMOnut enriched with oats, millet and 5% BAMOLP on 1 µm scale, (magnification 63.52 KX for a, b;2.53KX, c: 6.58KX).

4 *Water activity characteristics of Bambara groundnut-Moringa oleifera snack bar*

The water activity of the three BAMOnut (OB3, MB2, OMB5) is shown in Table 5.6. The water activity of the BAMOnut snack bars were 0.34, 0.57, and 0.58, respectively, and it is comparable to the 0.6 maximum standard requirement for RUTF. The BAMOnut-OB3 has the least water activity and it is significantly ($p < 0.05$) different from BAMOnut-MB2 and BAMOnut-OMB5. However, there was no significant difference between the water activity of BAMOnut-MB2 and BAMOnut-OMB5, their water activity compares with that of date and apricot bars (0.534-0.546), (Rehman *et al.*, 2012). Water activity is an indicator of available water in food, and it is a useful tool in the prediction of food quality and storage stability. The water activity of BAMOnut-MB2 and BAMOnut-OMB5 were higher due to the addition of cooked millet to both formulations, nevertheless, they are still within 0.6 max requirement for RUTF. The low water activity of the BAMOnut bars shows that there will be retarded growth of microorganisms in the

products, which implies good storage stability and long shelf life. BAMOnut snack bar enriched with oats and 3% BAMOLP (BAMOnut-OB3) could be selected as the best in terms of water activity and was also considered best in colour.

Table 5.6 Water activity of Bambara groundnut-*Moringa oleifera* snack bar

BAMOnut Variety	**Water Activity**
OB3	$0.34 \pm 0.01^{a.}$
MB2	0.57 ± 0.01^{b}
MMB5	0.58 ± 0.00^{b}

Mean values ± standard deviation of triplicate determination. Mean values in the same column followed by different letters are significantly ($p < 0.05$) different, BAMOnut: Bambara groundnut-*Moringa oleifera* snack bar, OB3: BAMOnut snack bar enriched with oats and 3% BAMOLP, MB2: BAMOnut snack bar enriched with millet and 2% BAMOLP, OMB5: BAMOnut snack bar enriched with oats, millet, and *5%* BAMOLP.

5 Textural Properties profile of BAMOnut Bar

Texture is an important parameter that is associated with a food product. It is the sensory manifestation of the structure of a food and reveals the mode that the structure responds to an applied force. Textural parameters tested were cut (shear) test, puncture test springiness, hardness, and gumminess (Table 5.7). BAMOnut-OB3 was significantly ($p < 0.05$) higher in cutting energy, puncture energy, hardness than BAMOnut-MB2 and BAMOnut-OMB5, but there was no significant difference in hardness of BAMOnut-MB2 and BAMOnut-OMB5. BAMOnut-OB3 was not springy, and there was no significant difference between BAMOnut-MB2 and BAMOnut-OMB5 in terms of springiness and gumminess. The higher hardness of BAMOnut-OB3 can be linked to its lower moisture content compared to BAMOnut-MB2 and BAMOnut-OMB5 in which cooked millets were added to the formulation.

The higher hardness of OB3 explains higher cutting and higher puncture energy, which can further be linked to the addition of roasted oats to the formulation compared to MB2 and OMB5 in which cooked wet millets were added. The bulkiness of the dry ingredient enhances proper absorption of the wet ingredients which results in low moisture content and a firmer product. It is therefore not surprising that MB2 and OMB5 were gummier, less cutting, and has less puncture strength, because of insufficient dry ingredients in the formulation.

Table 5.7 Textural profile of Bambara groundnut-*Moringa oleifera* snack bar

BAMOnut Bars	Cut (shear) N	Punture (N)	Springiness (mm)	Hardness (N)	Gumminess (N)
OB3	127.86 ± 41.90[a]	45.56 ± 9.41[a]	0.00 ± 0.00[a]	500.56 ± 0.47[a]	0.00 ± 0.00[a]
MB2	25 10 ± 1.98[b]	8.35 ± 1.88[b]	2.56 ± 0.24[b]	341.05 ± 21.14[b]	673.23 ± 26.72[b]
OMB5	18.13 ± 4.44[c]	2.68 ± 0.24[c]	3.19 ± 0.86[b]	300.24 ± 37.08[b]	642.38 ± 24.68[b]

Mean values ± standard deviation of triplicate determination. Mean values in the same column followed by different letters are significantly ($p < 0.05$) different, BAMOnut: Bambara groundnut-*Moringa oleifera* snack bar, OB3: BAMOnut snack bar enriched with oats and 3% BAMOLP, MB2: BAMOnut snack bar enriched with millet and 2% BAMOLP OMB5: BAMOnut snack bar enriched with oats, millet, and *5%* BAMOLP.

5.7.2 Proximate and amino acid composition of Bambara groundnut-*Moringa oleifera* snack bar

Amino acid profile and chemical composition of Bambara groundnut-*Moringa oleifera* (BAMOnut) snack bar are discussed in this section. The amino acid composition of BAMOnut-OB3 (BAMOnut Bar enriched with oats and 3% BAMOLP), BAMOnut-MB2 (BAMOnut Bar enriched with millets and 2% BAMOLP), and BAMOnut-OMB5 (BAMOnut Bar enriched with oats, millets, and 5% BAMOLP), are presented in Table 5.8. The total amino acids in BAMOnut-OM, BAMOnut-MB2, and BAMOnut-OMB5 were 15 65, 14.82, and 11.70 g/100 g respectively. The essential amino acid of the aforementioned BAMOnut bars were 38.47, 39.34, and 37.01% of the total amino acids, respectively. BAMOnut-OB3 and BAMOnut-MB2 snack bars were significantly ($p < 0.05$) higher in threonine, phenylalanine, histidine, valine, isoleucine, and leucine in comparison to BAMOnut-OMB5 (Figure 5.7). Tryptophan was lost during acid hydrolysis of the protein, hence it was absent in all the samples

The non-essential amino acid of BAMOnut-OB3, BAMOnut-MB2, and BAMOnut-OMB5 snack bars were 61.53, 60.66, and 62.99% of the total amino acids, respectively. Major non-essential amino acids observed were glutamine, arginine, and asparagine. Arginine was significantly higher in BAMOnut-OB3 and BAMOnut-MB2 than in BAMOnut-OMB5, meanwhile, arginine has been reported to prevent heart disease (Adebowale *et al.*, 2011). BAMOnut-OB3 and BAMOnut-OMB5 had significantly higher asparagine and alanine than BAMOnut-MB2. Glutamine was higher in BAMOnut-MB2 and BAMOnut-OMB5. Cysteine was not detected in the BAMOnut snack bar samples.

Amino acids are organic compounds, which are precursors of proteins; therefore, they influence the quantity and quality of protein (Fasuyi and Aletor, 2005; Moyo *et al.*, 2011; Alain Mune Mune *et al.*, 2016). Amino acids are categorised as essential and non-essential and vary according to animal species and their production system. They are indispensable in the production of enzymes, immunoglobins, hormones, growth, and repair of body tissues and form the structure of red blood cells (Brisibe *et al*., 2009). Furthermore, they play an important role in the formation of glucose, acting as a buffer when other precursors are in short supply. Amino acids are essential for the performance of specific functions in the body (Moyo *et al.*, 2011). BAMOnut-OB3 and BAMOnut-MB2 showed a better amino acid profile compared to BAMOnut-OMB5 (Table 5.8, Figure 5.7). Although there was no significant difference in some of the amino acids between BAMOnut-OB3 and BAMOnut-MB2, BAMOnut-OB3 had a higher content of phenylalanine, histidine, serine, glycine, glutamine, proline, and tyrosine. BAMOnut snack bar enriched with oats and 3% BAMOLP (BAMOnut-OB3) can therefore be considered to be the best in terms of amino acid profile, the same conclusion was made for colour and water activity.

Table 5.8 Amino acid composition of BAMOnut

	BAMOnut Bar		
Essential Amino acid (g /100 g)	OB3	MB2	OMB5
Threonine	0.87 ± 0.02[a.]	0.82 ± 0.02[a]	0.53 ± 0.01[b]
Methionine	0.26 ± 0.00	0.29 ± 0.00	0.14± 0.00
Phenyalanine	1.60 ± 0.02[a]	1.43 ± 0.02[b]	1.05 ± 0.01[c]
Histidine	0.57 ± 0.01[a]	0.50 ± 0.01[b]	0.37 ± 0.00[c]
Lysine	0.12 ± 0.00	0.22 ± 0.00	0.26 ± 0.00
Valine	0.77 ± 0.01[a]	0.75 ± 0.00[a]	0.57 ± 0.01[b]
Isoleusine	0.65 ± 0.01[a]	0.66 ± 0.01[a]	0.50 ± 0.01[b]
Leucine	1.18 ± 0.03[a]	1.16 ± 0.02[a]	0.91 ± 0.01[b]
Non-Essential Amino acid (g/100 g)	OB3	MB2	OMB5
Serine	1.08 ± 0.01[a]	0.96 ± 0.01[b]	0.71 ± 0.01[c]
Arginine	1.85 ± 0.03[a]	1.86 ± 0.04[a]	1.41 ± 0.01[b]
Glycine	1.03 ± 0.01[a]	0.97 ± 0.01[b]	0.64 ± 0.01[c]
Asparagine	1.13 ± 0.01[a]	1.25 ± 0.00[b]	1.13 ± 0.02[a]
Glutamine	2.40 ± 0.05[a]	2.12 ± 0.02[b]	2.09 ± 0.03[b]
Alanine	0.55 ± 0.01[a]	0.61 ± 0.01[b]	0.52 ± 0.01[a]
Proline	0.59 ± 0.01[a]	0.54 ± 0.01[b]	0.46 ± 0.01[c]
Tyrosine	1.00 ± 0.01[a]	0.68 ± 0.01[b]	0.41 ± 0.00[c]
TAA	15.65	14.82	11.70
TEAA	6.02	5.83	4.33
TNAA	9.63	8.99	7.37
%TEAA/TAA	38.47	39.34	37.01
%TNAA/TAA	61.53	60.66	62.99

Mean values ± standard deviation of triplicate determination. Mean values in the same row followed by different letters are significantly ($p < 0.05$) different, BAMOnut: Bambara groundnut-*Moringa oleifera* snack bar, OB3: BAMOnut snack bar enriched with oats and 3% BAMOLP, MB2: BAMOnut snack bar enriched with millet and 2% BAMOLP, OMB5: BAMOnut snack bar enriched with oats, millet, and *5%* BAMOLP. TAA: Total amino acid, TEAA: Total essential amino acid, TNAA: Total non-essential amino acid

2 Chemical composition of BAMOnut snack bar.

The chemical composition of BAMOnut-OB3 (BAMOnut Bar enriched with oats and 3% BAMOLP), BAMOnut-MB2 (BAMOnut Bar enriched with millets and 2% BAMOLP), and BAMOnut-OMB5 (BAMOnut Bar enriched with oats, millets, and 5% BAMOLP), are displayed in Table 5.9. The moisture contents of BAMOnut-OB3, BAMOnut-MB2, and BAMOnut-OMB5 are 4.9, 7.9, and 5.8% respectively. The moisture contents of the BAMOnut snack bars differed significantly ($p < 0.05$) from each other. The moisture content is higher than 2.5% required for RUTF, this may be due to the high moisture contents of some ingredients used in the formulation (8.0, 5.0, 4.7, 5.2, and 8.0% for oats, millet, BAMOLP, melon seed, and MOLP respectively). However the moisture content is comparable to the moisture content of snack bars reported by some researchers ((Sun-Waterhouse *et al.*, 2010; Rehman *et al.*, 2012)), and is lower than the granola experimental bar produced by Agbaje *et al*. (2016). Seeing that the moisture content of BAMonut is comparable to previous work on cereal bars and even lower than some, it can be concluded that BAMOnut snack bar will be shelf-stable because lower moisture content will enhance product stability. Formulation with millet had higher moisture because the millet was cooked and used wet. BAMOnut-OB3 has the least moisture content, and hence, can be stored for a long time without spoilage and low susceptibility to microbial spoilage.

The protein content of the BAMOnut snack bars were 14.1, 14.8, and 11.4% for BAMOnut-OB3, BAMOnut-MB2, and BAMOnut-OMB5, respectively, values were lower compared to the optimisation goal (24.0, 27.0, and 22.6%). There was no significant difference between the protein content of BAMOnut bar enriched with oats and 3% BAMOLP (BAMOnut-OB3), and from those enriched with millet and 2% BAMOLP (BAMOnut-MB2), but both were significantly ($p < 0.05$) different from BAMOnut-OMB5 in protein content. The highest protein content was observed at 2% (low concentration) of BAMOLP, in combination with a high concentration of both egusi and MOLP. The protein content decreased with increased concentration of BAMOLP, this could be due to synergistic effects of the other ingredients especially egusi and MOLP which decreased as BAMOLP increased. However, there was no significant difference in protein content when BAMOLP was added at low and medium concentrations. Nevertheless, the protein content increased with an increase in the concentration of *Moringa oleifera* leaf powder (MOLP). Olorode *et al* (2013) reported that the protein content of ‘ogi’ increased when MOLP was added to it, the same for supplementation of wheat flour with MOLP in bread (Sengev *et al.*, 2013). However, the addition of the Bambara groundnut-*Moringa oleifera* leaf protein complex (BAMOLP) to the formulation resulted in an insignificant increase in protein content because the formulation with the highest BAMOLP has the least protein content, but with a better amino acid profile. It could be because BAMOLP was added in smaller quantities,

Table 5.9 Chemical composition (g / 100 g) of BAMOnut Bar (OM, MM, and OMM)

Proximate (%)	OB3	MB2	OMB5	RUTF Requirement
Moisture	4.9 ± 0.2[a]	7.9 ± 0.6[c]	5.8 ± 0.1[b]	2.5
Protein	14.1 ± 0.9[a]	14.8 ± 0.9[a]	11.4 ± 0.5[b]	13 – 16% by weight
	12.04	13.78	10.45	10-12% total energy
Ash	2.06 ± 0.04[a]	3.04 ± 0.03 [b]	1.57 ± 0.01[c]	-
Fat	19.3 ± 1.2[a]	14.7 ± 0.9[b]	13.2 ± 0.2[b]	26 – 36% by weight
	37.16	30.79	27.22	45-60% total energy
Saturated	4.9 ± 0.4[a]	3.7 ± 0.1[b]	3.2 ± 0.1[c]	-
Monounsaturated	6.4 ± 0.7[a]	4.9 ± 0.1[b]	4.6 ± 0.1 [b]	-
Polyunsaturated	7.9 ± 0.9[a]	6.1 ± 0.1[b]	5.4 ± 0.1[b]	-
Carbohydrates	59.7 ± 1.1[a]	59.6 ± 0.4[a]	68.1 ± 0.3[b]	41 – 58
Energy (Kcal)	468.6 ± 10.7[a]	429.7 ± 3.2[b]	436.4 ± 0.84.[b]	520 – 550

Mean values ± standard deviation of triplicate determination. Mean values in the same row followed by different letters are significantly ($p < 0.05$) different, BAMOnut: Bambara groundnut-*Moringa oleifera* snack bar, OB3: BAMOnut snack bar enriched with oats and 3% BAMOLP, MB2: BAMOnut snack bar enriched with millet and 2% BAMOLP, OMB5: BAMOnut snack bar enriched with oats, millet, and *5%* BAMOLP.vRUTF: Ready-to-use therapeutic food

hence not sufficient to produce a significant effect. As per RUTF requirement, a protein-calorie contribution is expected to be 10 -12% of the total energy, all the three variations of the BAMOnut snack bar met this standard, 12.0, 13.8, 10.4% total energy for BAMOnut-OB3, BAMOnut-MB2, and BAMOnut-OMB5 respectively.

The fat content of the BAMOnut snack bars were 19.3, 14.7, and 13.2% for BAMOnut-OB3, BAMOnut-MB2, and BAMOnut-OMB5 respectively. There was no significant ($p > 0.05$) difference between the fat content of BAMOnut snack bar enriched with millet and 2% BAMOLP (BAMOnut-MB2), and from those enriched with oats, millet, and 5% BAMOLP (BAMOnut-OMB5), but both were significantly ($p < 0.05$) different from BAMOnut-OB3 in fat content. The difference in the fat content could be as a result of cooked millets, which are low in fat (1.7% fat content) added to the formulations of BAMOnut-MB2 and BAMOnut-OMB5, while oats (6.9% fat content) was added in the formulation of BAMOnut-OB3. The fat content of RUTF is expected to be 45–60% total energy, 37.0, 30.8, and 27.2% total energy was obtained for BAMOnut-OB3, BAMOnut-MB2, and BAMOnut-OMB5, respectively. A reduction in the concentration of honey added to the formulation from 45% to 30%, and an increase in canola oil from 15% to 30% may likely increase the fat content of the BAMOnut snack bar. This reasoning is because honey mainly contributed to carbohydrates content which was higher than the required for RUTF in all three variations.

The ash contents of BAMOnut bars were significantly ($p < 0.05$) different from each other. BAMOnut-MB2 (3.04%) has the highest content of ash, followed by BAMOnut-OB3 (2.06), and BAMOnut-OMB5 (1.57). It was observed that the ash content increased with the increased concentration of MOLP in the formulations (Table 5.1). Several studies revealed that *Moringa oleifera* leaf powder is high in ash contents which ranges from 4.6-10.9% (Elmoneim *et al.*, 2007; Busani *et al.*, 2011; Oluduro, 2012; Sengev *et al.*, 2013; Sodamade and Adeboye, 2013; Teixeira *et al.*, 2014; Gebregiorgis Amabye, 2015; Sohaimy *et al.*, 2015). Ash content is an indication of the mineral matter present in the food substance (Sodamade and Adeboye, 2013).

There was no significant ($p > 0.05$) difference between the carbohydrate composition of BAMOnut-OB3 and BAMOnut-MB2 but both differ ($p < 0.05$) significantly from BAMOnut-OMB5. The difference is probably due to a combination of oats and millet used in BAMOnut-OMB5. The carbohydrate content of BAMOnut snack bars is higher than the recommended value for RUTF (41-58%) but is comparable to previous research work on cereal bars.

The energy values of the BAMOnut snack bar were 468.6, 429.4, and 436.4 Kcal for BAMOnut-OB3, BAMOnut-MB2, and BAMOnut-OMB5, respectively. The energy content of BAMOnut-OB3 was significantly ($p < 0.05$) higher than the content of BAMOnut-MB2 and BAMOnut-OMB5. However, there was no significant ($p > 0.05$) difference between the energy content of BAMOnut-MB2 and BAMOnut-OMB5. The difference in energy is due to the difference in fat content of the BAMOnut snack bars, fat provides a high coefficient of nutritional

values (9 Kcal) compared to protein and carbohydrate which provides a lower coefficient of nutritional values (4 Kcal). The energy content of BAMOnut snack bars is lower compared to the recommended value (520-550) Kcal for RUTF, but comparable to the energy value of cereal bars in previous studies (Agbaje *et al.*, 2016). Therefore, BAMOnut snack bars could be classified as a high-calorie snack bar.

5.7.3 Sensory

The demography of the panellist who partook in the bench-top sensory is shown in Table 5.10. The ten (10) panellists comprised 30% males and 70% females, 20% were staff, and 80% were students. The age categories of the panellists are as follows; less than 20 years (20%), within 20 – 29 years (40%), within 30 – 39 years (30%), and greater than 40 years (10%). Sensory scores for sensorial parameters and overall acceptability of BAMOnut snack bars are presented in Figure 5.7. The panellists mean ratings for appearance, colour, aroma, taste, texture, and overall acceptability of BAMOnut-OB3, BAMOnut-MB2 and BAMOnut-OMB5 were illustrated in Figure 5.7.

Table 5.10 Demography of Assessors

Item	**Category**	**Frequency (%)**
Gender	Male	3 (30)
	Female	7 (70)
Age	Less than 20	2 (20)
	< 20-29	4 (40)
	30-39	3 (30)
	40 & above	1 (10)
Staff or student	Staff	2 (20)
	Student	8 (80)
International student	Yes	4 (40)
	No	6 (60)

Numbers are frequency and percentage in a bracket

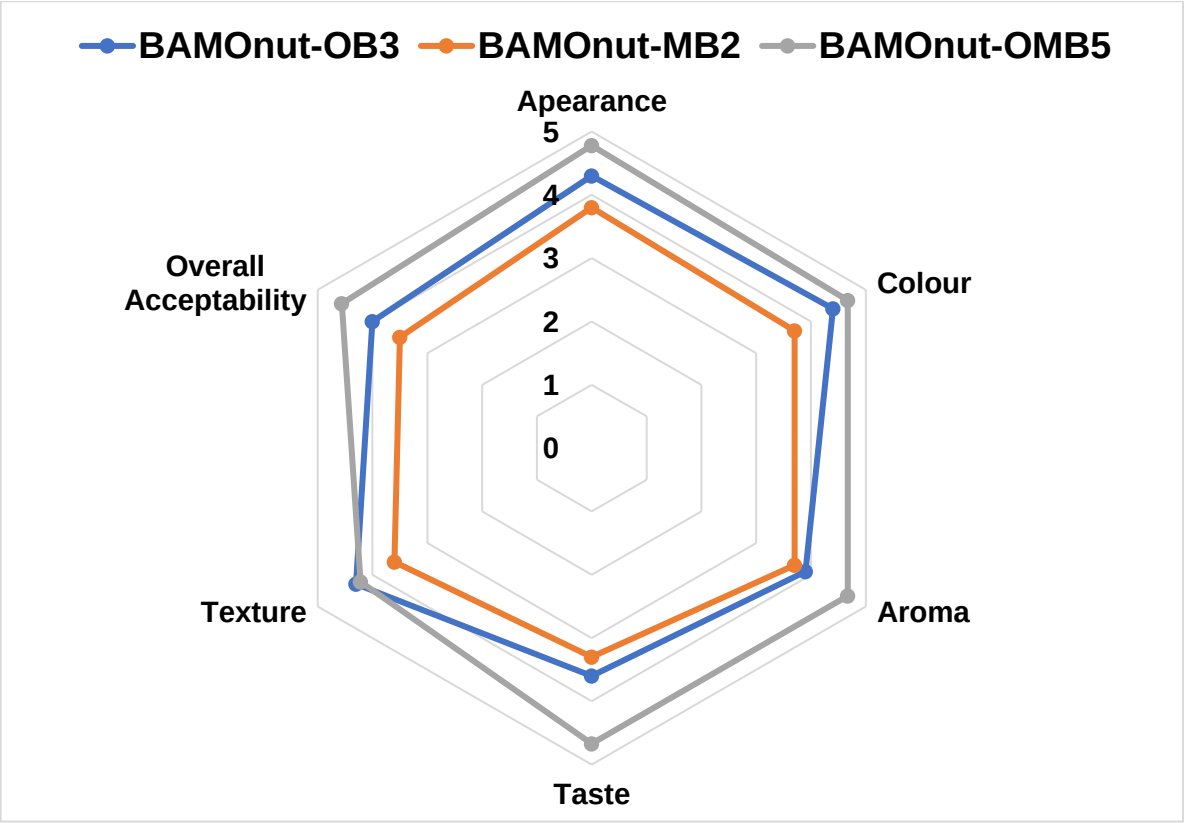

Figure 5.7 Spider plot showing sensory scores of Bambara groundnut-*Moringa oleifera* snack bars (BAMOnut)

Two of the BAMOnut snack bars (BAMOnut-OB3 and BAMOnut-OMB5) were not significantly ($p > 0.05$) different for appearance, colour, and aroma but both were significantly ($p < 0.05$) different to BAMOnut-MB2. The appearance of BAMOnut-OB3 and BAMOnut-OMB5 (4.30, 4.78) were significantly ($p < 0.05$) higher as compared to that of BAMOnut-MB2 (3.80). The colour of BAMOnut-OB3 and BAMOnut-OMB5 (4.40, 4.67) were significantly ($p < 0.05$) higher in comparison to BAMOnut-MB2 (3.70). The panellist described the colour of BAMOnut-MB2 as very dark. The aroma of BAMOnut-OB3 and BAMOnut-OMB5 (3.90), (4.67) were significantly ($p < 0.05$) higher as compared to that of BAMOnut-MB2 (3.70). The bar (BAMOnut-MB2) in which millet and 2% BAMOLP was incorporated had lower ratings for appearance, colour, and aroma due to a high concentration (80 g) of MOLP used in the formulation. A similar observation was reported for MOLP supplementation in wheat bread by Sengev *et al*. (2013).

BAMOnut-OB3 and BAMOnut-MB2 were not significantly ($p > 0.05$) different in terms of taste and overall acceptability. The taste of BAMOnut-OB3 and BAMOnut-MB2 was significantly ($p < 0.05$) rated lower (3.60, 3.30) compared to that of BAMOnut-OMB5 (4.67). Some of the panellists described the taste of BAMOnut-MB2 as slightly bitter while some described it as a mild burnt taste which is due to the high concentration of MOLP in the

formulation. Statistically, there was no significant difference in the texture of all three variants of the BAMOnut snack bar. The overall acceptability of BAMOnut-OB3 and BAMOnut-MB2 was significantly ($p < 0.05$) lower compared to BAMOnut-OMB5 (4.56), the difference can be attributed to the high concentration of MOLP in the formulations. The panellist described BAMOnut-OB3 as too sweet, nice, and crunchy, and a little bit hard, BAMOnut-OMB5 as too sweet, soft, and crunchy and delicious. While BAMOnut-MB2 was described as having a very dark colour, mild burnt taste, slightly bitter taste, bitter, strong aroma, soft and crumbly.

5.7.4 The principal component of the Bambara groundnut-*Moringa oleifera* snack bar

Principal component analysis (PCA) on the physical and proximate properties of Bambara groundnut-*Moringa oleifera* snack bar data is presented in Figure 5.8. The PCA is a statistical method with which a large number of variables are reduced into few to describe the greatest variance in the analysed data. The PCA was used to visualize the variation in the properties among the three BAMOnut snack bars. The first and second principal components represented 78.8% and 21.2% of the observed variation (100% in total). PC1 separated BAMOnut- bars, with the BAMOnut-MB2 and BAMOnut-OMB5 on the top right and bottom right respectively, and the BAMOnut-OB3 is at the middle by the right side. BAMOnut-OB3 is more related with high fat (mono and poly fat), cutting, energy, and lightness, BAMOnu-MB2 with ash content and moisture, and BAMOnut-OMB5 with carbohydrate. Yellowness and protein content can be attributed to both OB3 and MB2. The difference in fat content of BAMOnut-OB3 compared to BAMOnut-MB2 and BAMOnut-OMB5 may be due to the high content of fat in oats (8%) which was incorporated, while 4.8% of fat in millet. High-fat content leads to high energy. The lightness of BAMOnut-OB3 could be as a result of less concentration of MOLP in the formulation compared to BAMOnut-MB2, and variation of ingredients when compared to OMB5. The ash content which is an indication of mineral was higher in BAMOnut-MB2 due to the higher concentration of MOLP in the formulation compared to BAMOnut-OB3 and BAMOnut-OMB5, and moisture content was higher due to the added cooked millet in the formulation. BAMOnut-OB3 and BAMOnut-MB2 could be considered as better formulations compared to BAMOnut-OMB5. The moisture, energy, and fat content of BAMOnut-OB3 are closer to the required standard for RUTF compared to the content in BAMOnut-MB2, and the colour of BAMOnut-OB3 which is an important attribute for consumer acceptability of a food product is better. Although BAMOnut-MB2 was high in ash content, the moisture content was high, and it will be safer to choose a formulation with a lower moisture content to guarantee good shelf life and good quality product. BAMOnut-OB3 could therefore be considered as the best formulation for the BAMOnut-snack bar, which was also confirmed with the water activity in section 5.6.1 and amino acid content in section 5.6.2.

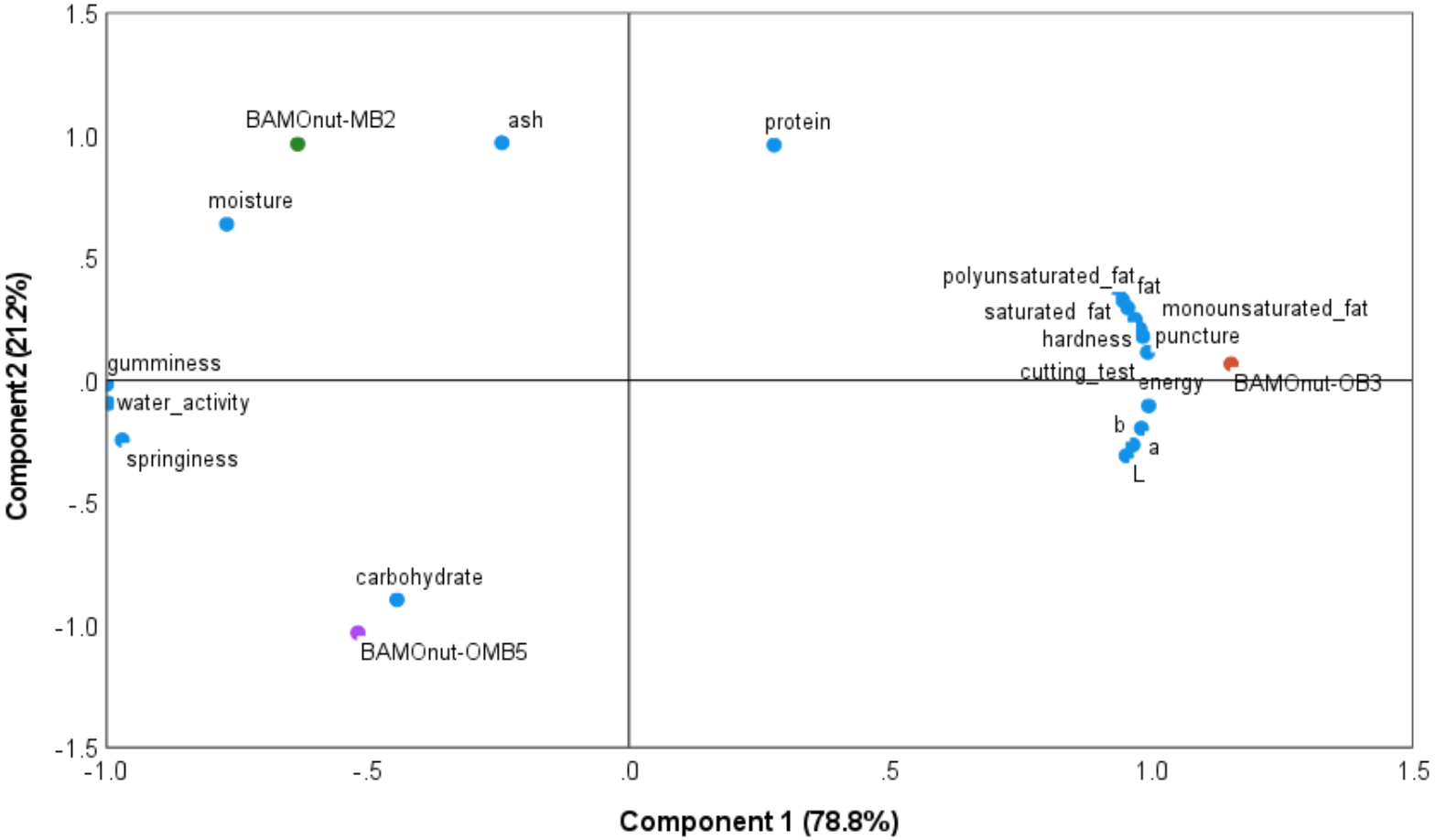

Figure 5.8 Principal component of Bambara groundnut-*Moringa oleifera* snack bar.

5.8 Conclusion

The study confirmed that ready-to-use therapeutic food (RUTF) that will meet WHO requirements can be produced from legumes and cereals. BGN and *Moringa oleifera* leaf powder with the addition of egusi, oats, and millets were successfully used to produce snack bars of acceptable sensory scores and nutritional value. BAMOnut snack bar produced with oats can be considered as the best formulation. The protein content of the BAMOnut snack bar ranges from 11.4 -14.8%. The protein content of BAMOnut-snack bars (OB3 and MB2) is comparable to 13–16% required for RUTF, which implies that RUTF can be produced without the addition of the full milk powder used as standard RUTF ingredient. The low water activity of the bars is an indication of a low risk of microbial growth. The BAMOnut snacks may serve as an alternative RUTF for severely acutely malnourished children and also as nutritious snacks for adults (Weber *et al.*, 2017).

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CHAPTER SIX

GENERAL SUMMARY AND CONCLUSION

Bambara groundnut-*Moringa oleifera* leaf protein complex was produced in this study. The study aimed to investigate the nutritional and functional properties of the BGN-*Moringa oleifera* leaf protein complex and its application in ready-to-use therapeutic food (RUTF).
The following objectives were achieved in this study:

1. Extraction of protein isolate from Bambara groundnut (BGN) flour and *Moringa oleifera* leaf powder (MOLP) to produce a protein complex from the two protein isolates.

This objective was achieved as BGNPI and MOLPI were successfully extracted from BGN flour and MOLP. BGNPI was extracted by isoelectric precipitation at pH 9 and pH 5 and MOLPI by heat application at 90^{o}C. A preliminary analysis on the behaviour of the protein isolates (BGNPI and MOLPI) in solution was carried out to determine their optimal mix for the production of a protein complex from both protein isolates. Therefore, the interaction of BGNPI and MOLPI in a solution was investigated and a protein mixture with 9% BGNPI and 2% MOLPI was established as the optimal mix for the protein isolates The protein complex (BAMOLP) was produced. BGNPI and MOLPI isolate in a water system are most stable when the concentration of BGNPI is at the upper limit (9).

2. Evaluation of the nutritional and functional characteristics of the BGN protein isolates (BGNPI) and MOLP protein isolates (MOLPI).

The second objective was achieved by characterizing the nutritional and functional properties of BGNPI and MOLPI. The proximates composition of the two isolates differs, BGNPI exhibits a higher protein and fat content compared to MOLPI, while MOLPI has a higher content of ash and carbohydrate. The difference in protein and fat content is related to extraction methods. Loss of protein during the extraction of MOLPI was inevitable leading to lower yield and incomplete extraction of fat from BGN flours results in higher fat content of BGNPI. Glutamine and asparagine are the major non-essential amino acid in the protein isolates. The content of lysine, asparagine, and glutamine in BGNPI was higher than in MOLPI. The protein isolates will be a suitable functional food ingredient in value-added products. They are good sources of lysine which will promote their use as a supplementary protein in the formulation of cereal products that are deficient in lysine. The oil absorption capacity of BGNPI was higher than that of MOLPI, whereas MOLPI had higher water absorption compared to BGNPI. The hypothesis that the nutritional and functional properties of BGNPI and MOLPI will be different was accepted.

3. Evaluation of the nutritional and chemical properties of Bambara groundnut-*Moringa oleifera* leaf protein isolates (BAMOLP).

The third objective was achieved by characterizing the nutritional and functional properties BAMOLP. The BAMOLP exhibits a better amino acid profile and chemical properties than its precursors. Glutamine and asparagine are the major non-essential amino acid in the BAMOLP. The content of lysine, asparagine, and glutamine in BAMOLP was higher than in BGNPI and MOLPI. BAMOLP was higher in methionine, phenylalanine, and histidine compared to whey, pea, brown rice, soy, hemp, and wheat protein. The amino acid content of BAMOLP was higher in threonine, phenylalanine, lysine, and leucine when compared to FAO/WHO reference pattern. BAMOLP will be a suitable functional food ingredient in value-added products. BAMOLP is a good source of lysine which will promote its use as a supplementary protein in the formulation of cereal products that are deficient in lysine. Conclusively, complementing BGNPI and MOLPI was successful as BAMOLP showed an improvement over its precursors. The hypothesis that there will be differences in nutritional and functional properties of the BGN-*Moringa* protein complex compared to its individual isolates was accepted.

4. Production of a ready-to-use therapeutic bar using BGN-*Moringa oleifera* leaf protein complex to establish its nutritional and proximate characteristics, especially in comparison to standard RUTF requirement.

The ready-to-use therapeutic bar using the standard RUTF requirement was carried out as reported in this thesis. This objective was achieved by using a combination of some legumes and cereals in different proportions to enrich the nutritional and proximate properties of the snack bar. The BAMOnut snack bar produced was comparable to the standard RUTF in terms of protein, carbohydrate, and energy content. The hypothesis that BAMOLP will be suitable for the production of a ready-to-use therapeutic bar and that the bar will meet protein requirement for RUTF set by WHO was accepted.

The following conclusions can be drawn from this study:

1. Nutritional and functional properties of BGNPI extracted from BGN seeds make it suitable as a functional food ingredient.
2. Nutritional and functional properties of MOLPI extracted from *Moringa oleifera* leaf powder make it suitable as a functional food ingredient.
3. Protein complex (BAMOLP) produced from BGNPI and MOLPI is a functional food ingredient to produce a ready-to-use therapeutic bar.
4. An alternative ready-to-use therapeutic snack bar (BAMOnut) that is comparable to the nutritional profile of the standard ready-to-use therapeutic food (RUTF) can be produced from BAMOLP, MOLP, egusi, oats, and millets
5. A ready-to-use therapeutic snack bar produced using BAMOLP, MOLP, egusi, oats, and millets were acceptable to consumers.

Outputs from this study include the following:

1. Manuscript

Adewumi O. O., Felix-Minnaar J. V., Jideani V. A. (2021). Functional properties and amino acid profile of Bambara groundnut and *Moringa oleifera* leaf protein complex. Manuscript accepted for publication in MDPI, processes.

2 Conference Proceedings

Adewumi O. O., Felix-Minnaar J. V., Jideani V. A. (2021). Functional properties and amino acid profile of Bambara groundnut and *Moringa oleifera* leaf protein complex. 24th South African Association of Food Science and Technology (SAAFoST) Biennial International Virtual Congress 2021, Durban, South Africa, 20 - 22 September 2021. (Poster presentation).

Adewumi O. O., Felix-Minnaar J. V., Jideani V. A. (2021). Phase behaviour of *Vigna subterranea* and *Moringa oleifera* protein isolates in aqueous solution.CPUT Postgraduate Conference, Bellville, Cape Town, South Africa, 30 November 2021 (Poster presentation).

Appendices

Appendix A: Article submitted MDPI, processes titled; Functional properties and amino acid profile of Bambara groundnut and *Moringa oleifera* leaf protein complex

Dear Mrs. Adewumi,

Thank you very much for uploading the following manuscript to the MDPI submission system. One of our editors will be in touch with you soon.

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Type of manuscript: Article
Title: FUNCTIONAL PROPERTIES AND AMINO ACID PROFILE OF BAMBARA GROUNDNUT AND
MORINGA OLEIFERA LEAF PROTEIN COMPLEX
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Appendix B: Approved ethic clearance

Cape Peninsula University of Technology

Statement of Permission

Data/Sample collection permission is not required for this study.

Surname & name	Olawumi Oluwakemi Adewumi
Student Number	210237376
Degree	Master of Food Science and Technology
Title	Nutritional and functional properties of Bambara groundnut and Moringa leaf protein complex in a Ready-to-use therapeutic food (RUTF)
Supervisor	Professor V.A. Jideani
FRC Signature	
Date	25/9/2018

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Reference number: 210237376

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 **Olawumi Oluwakemi Adewumi** for research activities related to the: **Master of Food Science and Technology** at the Cape Peninsula University of Technology requires / **does not** require ethical clearance.

Proposed title of dissertation/ thesis:	Nutritional and functional properties of Bambara groundnut and Moringa leaf protein complex in a Ready-to-use therapeutic food (RUTF)

Comments (Add any further comments deemed necessary, eg permission required)

Full/unconditional approval
Research activities are restricted to those detailed in the research proposal.

Signed: Chairperson: Research Ethics Committee	**Date** 25/9/2018

Appendix C: Book of abstracts – Research outputs presented at National conference proceedings.

1. Adewumi A: O., Felix-Minnaar J. V., Jideani V. A. (2021). Functional properties and amino acid profile of Bambara groundnut and *Moringa oleifera* leaf protein complex. 24th South African Association of Food Science and Technology (SAAFoST) Biennial International Virtual Congress 2021, Durban, South Africa, 20 - 22 September 2021. (Poster presentation).

2. Adewumi A: O., Felix-Minnaar J. V., Jideani V. A. (2021). Phase behaviour of *Vigna subterranea* and *Moringa oleifera* protein isolates in aqueous solution. CPUT Postgraduate Conference, Bellville, Cape Town, South Africa, 30 November 2021 (Poster presentation).