Cape Peninsula University of Technology

FUNCTIONAL PROPERTIES OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* (L.) VERDC.) NON-STARCH POLYSACCHARIDES IN MODEL AND FOOD SYSTEMS

by

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DECLARATION

I, **Claudine Florett Diedericks**, declare that the contents of this thesis represent my own original work, and that the 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.

C.F. Diedericks	**31 October 2014**
Signed	**Date**

ABSTRACT

The aim of this study was to evaluate bambara groundnut [BGN] non-starch polysaccharides [NSP] subject to the incorporation into model and food systems with a view to establish their functional and physicochemical properties.

BGN insoluble dietary fibre [BGNIF] and soluble dietary fibre [BGNSF] were successfully extracted from four varieties (black-eye: BLE, red: RED, brown: BRN and brown-eye: BRE). Physicochemical properties evaluated revealed the high bulk density of all BGNIF and BGNSF varieties, which could contribute to cost-effective packaging. The microstructures of BGNIFs were irregular in shape with different sizes. The colour parameters (lightness, redness, yellowness, chroma and hue angle) differed significantly [$p \leq 0.05$] across all BGNIF and BGNSF varieties; and indicated a yellowish-red colour for BGNIFs and a light yellow colour for BGNSFs. Negligible amounts of condensed tannins [CT] were found in BGNIFs (0.014 – 0.160 $mg.g^{-1}$). Higher amounts polyphenols [PP] were present in BGNSFs (45.42 – 55.90 $mg.g^{-1}$ gallic acid equivalents [GAE]) compared to the amount PP in BGNIFs (6.14 – 15.56 $mg.g^{-1}$ GAE). Major sugars identified were arabinose/galactose, xylose and mannose in BGNIFs, and xylose and mannose in BGNSFs. The functional properties evaluated revealed high swelling capacity of BGNIFs (6.37 – 7.72 $ml.g^{-1}$) and no significant [$p > 0.05$] difference in water retention capacity. Fat absorption capacity ranged from 1.38 – 1.52 g $oil.g^{-1}$ dry weight for BGNIFs and 4.04 – 4.55 g $oil.g^{-1}$ dry weight for BGNSFs. Variability in BGNIF (91.2%) and BGNSF (79.4%) physicochemical and functional properties could both be explained by two principal components (BGNIF component 1: PP, redness, yield; and component 2: xylose, yellowness and chroma; BGNSF component 1: yellowness, chroma, mannose content; and component 2: redness, fat absorption and fructose content).

Following an IV optimal mixture design, an optimum white bread formulation was obtained using 59.5% water, 4.3% yeast and 8.5% BGNIF. Bread enriched with the four BGNIF varieties (BLE, RED, BRN and BRE) were tested for several physicochemical properties. Significant [$p \leq 0.05$] differences existed between the control and BGNIF enriched loaves for crumb grain characteristics (including pore area distribution, feret angle, circularity, roundness and aspect ratio). Specific loaf volume of BGNIF enriched loaves ranged from 3.33 – 3.85 $ml.g^{-1}$ and were significantly [$p \leq 0.05$] lower compared to the control bread (4.16 $ml.g^{-1}$). Favourable texture characteristics obtained with the BGNIF enriched breads were lower hardness, chewiness and gumminess compared to the control loaf. Crust and crumb colour parameters (lightness, redness, yellowness, chroma and hue angle) were significantly [$p \leq 0.05$] different across all loaves. BRE BGNIF bread (3.43 ± 0.20) had the significantly [$p \leq 0.05$] lowest crumb colour difference compared to the control bread; whilst BRN (1.72 ± 0.42) and BRE (2.44 ± 0.78) loaves had the lowest significant [$p \leq 0.05$] crust

colour difference compared to the control. Favourable chemical properties were the high total dietary fibre [TDF] (7.14 – 8.33%) content of all BGNIF enriched loaves compared to the control loaf (4.96%). Significant [$p \leq 0.05$] differences were also observed for some loaves for moisture content, condensed tannins and polyphenol content. Variability in bread physicochemical properties was differentiated by three components (component 1: bread textural properties; component 2: specific loaf volume and bread lightness; component 3: crumb colour parameters) which accounted for a cumulative variation of 92.8%. All bread loaves were also sensorially acceptable as rated moderately like to like very much (>3 rating on a 5-point hedonic scale) by consumers for all parameters (appearance, crust and crumb colour, aroma, taste, texture and overall acceptability) evaluated.

Furthermore, brown BGNSF was tested for stabilising effects in an orange beverage emulsion. BGNSF and orange oil were varied at two levels each based on a 2^2 augmented factorial design and the effects determined on the equilibrium backscattering [BS] flux as emulsion stability indicator. The BS profiles which resulted from the Turbiscan stability analysis revealed flocculation at low rates as the major destabilisation mechanism. The optimal formulation producing a stable emulsion was identified as low oil (6%) and high BGNSF (30%) concentrations. The objective of this study was therefore achieved and showed that positive physicochemical and functional properties are associated with BGNIF and BGNSF from black-eye, red, brown and brown-eye varieties. Furthermore, the incorporation of BGN fibres in white bread and a beverage emulsion was shown to contribute positive technological properties in these systems.

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DEDICATION

For my parents, Joseph and Josephinus Diedericks; mere words cannot express my gratitude for your unwavering support, love and encouragement. To my brother, Xavier Diedericks, I appreciate you. My family and friends, your support and belief in me has not gone unnoticed, I thank you. Claudgen Swarts, thank you for (being my) Eccles. 4:9-10.

Philippians 4:13
I can do all things through Christ which strengtheneth me.

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Language and style used in the thesis are in accordance with the requirements of the International Journal of Food Science and Technology. The thesis represents a compilation of manuscript where each chapter is an individual entity and some repetitions between chapters have, therefore, been unavoidable.

GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
AACC	American Association of Cereal Chemists
ADF	Acid detergent fibre
ANOVA	Analysis of variance
BD	Bulk density
BGN	Bambara groundnut
BGNIF	Bambara groundnut insoluble dietary fibre
BGNSF	Bambara groundnut soluble dietary fibre
BLE	Black-eye
BRE	Brown-eye
BRN	Brown
BS	Backscattering
CATPCA	Categorical principal component analysis
CT	Condensed tannins
DPP	Directorate Plant Production
FA	Fat absorption
FAO	Food and Agriculture Organisation
IDF	Insoluble dietary fibre
MANOVA	Multivariate analysis of variance
NDF	Neutral detergent fibre
NS	Neutral sugars
NSP	Non-starch polysaccharides
PCA	Principal component analysis
PP	Polyphenolics
RED	Red
SC	Swelling capacity
SDF	Soluble dietary fibre
SEM	Scanning electron microscope
SLV	Specific loaf volume
SSPS	Soybean soluble polysaccharide
STEM	Science, technology, engineering and mathematics
TDF	Total dietary fibre
UA	Uronic acids
WRC	Water retention capacity

CHAPTER ONE
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Bambara groundnut (*Vigna subterranea* (L.)Verdc.) [BGN] is an easy-to-cultivate legume seed which is widely grown throughout tropical Africa, Indonesia, Malaysia, India, Sri Lanka, Central and South America and some parts of Northern Australia (Eltayeb *et al.*, 2011, De Kock, 2013). BGN originated in West Africa with its name derived from the Bambara tribe who now lives in Mali (Nwanna *et al.*, 2005). Considered as one of the main attributes of BGN is its tolerance of poor soils and drought, as well as its ability to yield in conditions in which groundnut fails completely. As a result of this crop being cultivated by women, the terms "the groundnut of the women" and "a poor man's food" have become synonymous with BGN (Brough *et al.*, 1993). BGN which is primarily grown for its edible seeds are lower in oil content compared to groundnuts, but higher in protein and carbohydrates. The seeds may be consumed fresh, grilled or it may be soaked and boiled before consumption (Brough *et al.*, 1993). More recently, the functional properties of BGN flour, protein and starch fractions have been investigated, as a means to better utilisation of this underutilised crop in food applications (Adebowale *et al.*, 2002; Adebowale & Lawal, 2004; Lawal *et al.*, 2007; Sirivongpaisal, 2008; Yusuf *et al.*, 2008; Eltayeb *et al.*, 2011). Compared with other legume seeds, the seeds from BGN are a relatively good source of dietary fibre (Tanya *et al.*, 1997); however no evidence could be found for the isolation and investigation of its dietary fibre fractions.

Polysaccharides, a term synonymous with gums and hydrocolloids, represent the form of at least 90% of carbohydrates available in nature (BeMiller, 2003). The inclusion of polysaccharides in food products is of importance, as they perform several functions such as gelling, foams and emulsions stabilisation and thickening (Nakamura *et al.*, 2006). The only polysaccharides digestible by humans are the starch polymers. Recognised as one of the most abundant food hydrocolloids, starch can be found in all parts of plant materials such as seeds, leaves and tubers. The non-starch polysaccharides on the other hand are those polysaccharides other than starch which are non-digestible, with dietary fibre comprising the major portion of this group (BeMiller, 2003).

As defined by the American Association of Cereal Chemists (AACC, 2001) dietary fibre is "the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation". Based on its

solubility in water, dietary fibre can be classified as insoluble dietary fibre or soluble dietary fibre (Elleuch *et al.*, 2011). These fractions are incorporated into food products for various technological (water holding capacity, gel-forming capacity, antioxidant capacity, etc.) and physiological (laxative, reduction of blood glucose, etc.) reasons (Elleuch *et al.*, 2011).

Legumes are rich in dietary fibre, and are therefore an important food source for the isolation of this heterogeneous mixture of polysaccharides. Research also reveals the superior technological properties of legume fibres, but despite these observations the only legume fibre fractions commercially available are those from peas (Meuser, 2001; Wang & Toews, 2011). There is thus a need to explore more legumes as sustainable dietary fibre food sources, and by highlighting the potential of one underutilised legume (i.e. BGN) the economic status of such crops could be positively changed.

1.2 Statement of Research Problem

Dietary fibre is important in the development of highly demanded functional and value-added foods, due to its beneficial role in health and physiology and the distinct functional properties it imparts in food products. As a result, the market for products and ingredients rich in dietary fibre has grown largely; hence there is the need to obtain new dietary fibre food sources for use in the food industry. Dietary fibre is found in several important food sources, with legumes being a rich source for isolation of this heterogeneous mixture of polysaccharides (Tharanathan & Mahadevamma, 2003). Research has been conducted on the dietary fibre fractions in several major crops such as peas, chickpeas, beans and lentils; however, several underutilised legume species like BGN remain to be investigated. Several studies also focussed on investigating the starch fractions of BGN seeds to highlight the potential value of BGN as sustainable food security crop (Adebowale *et al.*, 2002; Sirivongpaisal, 2008; Afolabi, 2012; Gabriel *et al.*, 2013). However, no information to date is available on the dietary fibre fractions from BGN. The fractionation of BGN into dietary fibre fractions could therefore address the need and fill the gap in the market for better utilisation of this under-valued crop, and as a result increase its market value.

1.3 Statement of the Objectives

1.3.1 Broad objective

The aim of this study was to evaluate BGN non-starch polysaccharides [NSP] subject to the incorporation into model and food systems with a view to establish their functional and physicochemical properties.

1.3.2 Specific objectives

The specific objectives of this study included the following:

i. Isolation and characterisation of BGN NSP.
ii. Determination of the functional properties of insoluble and soluble NSP from BGN in model systems.
iii. Establish the functionality of BGN insoluble dietary fibre [BGNIF] in white bread.
iv. Establish the ability of BGN soluble dietary fibre [BGNSF] to stabilise a beverage emulsion.

1.4 Hypotheses

The following hypotheses were tested in this study:

i. The NSP isolated from the four BGN varieties will differ from each other in their functionality in model systems.
ii. Insoluble dietary fibre from different BGN varieties will differ in their functionality in white bread.
iii. Soluble dietary fibre from BGN will stabilise a beverage emulsion.

1.5 Delineation

Only four varieties of BGN– black-eye, red, brown and brown-eye, were evaluated.

1.6 Significance of the Research

With the current situation of chronic food insecurity in sub-Saharan Africa, underutilised legumes have been highlighted as a means of building food security through increased agricultural productivity and food use (United Nations Development Programme, 2012). Research on the dietary fibre fractions of BGN would thus have significant socioeconomic implications. Economically, a new source of dietary fibre would be available to the food industry which could lead to export of the BGN crop and/or fibres, and eventually lead to an increase of GDP. A greater demand for BGN by the food industry would lead to higher production levels of BGN and consequent positive implications for farmers. BGN are typically grown by women farmers (Brough *et al.*, 1993); an increasing demand for BGN seeds would thus lead to the empowerment of women in rural areas and improve the livelihood of families. Furthermore, this research will lead to a higher degree which positively impacts the education output of South African women in the field of STEM (science, technology, engineering and mathematics).

1.7 Expected Outcomes, Results and Contributions of the Research

The following were expected from this study:

i. Establishing a new source of dietary fibre fractions to be used in the food industry.
ii. BGNSF as alternative/readily available source for the stabilisation of beverage emulsions.
iii. Successfully highlighting the potential of BGN as important food crop.
iv. Submission of at least one research article for publication in an accredited journal; thereby contributing new knowledge to the field of food science and technology.
v. Presenting the research output at minimum one local and one international conference.

1.8 Thesis Overview

This thesis was compiled in article format, consisting of six chapters where each research chapter is a complete entity. Chapter one introduces the research overview, including the objectives, research problem and anticipated significance of the research. Chapter two is the literature review which expands on the background of the research topic. The definition, extraction and physiological and technological role of dietary fibre were reviewed; followed by the potential of specifically legume fibres. Finally, the rich nutritional profile and the current research to date on the technological properties of BGN were highlighted, and the potential of dietary fibre fractions from the seeds were discussed. Chapter three is the first research chapter, focussing on the isolation and characterisation of BGN soluble and insoluble dietary fibres. In this chapter the BGN fibres (black-eye, red, brown and brown-eye insoluble and soluble fibres) were extracted and evaluated for some physicochemical and functional properties. Chapter four is the second research chapter and focussed on the functionality of BGNIF in white bread. BGNIF from four varieties (black-eye, red, brown and brown-eye) were incorporated into an optimal white bread formulation, and the bread physicochemical properties evaluated and compared to the control white bread. The consumer acceptance of the bread was also evaluated. Chapter five is the final research chapter and focussed on the effect of BGNSF on stability of orange beverage emulsion. Brown BGNSF and orange oil were incorporated at different concentrations in six formulations as obtained by a 2^2 factorial design. Emulsions were tested for stability and the best emulsion formulation identified. Chapter six is a summary of the research, focussing on the important findings and final conclusions of the study.

References

AACC (2001). The definition of dietary fibre (Report of the Dietary Fibre Definition Committee to the Board of Directors of the AACC). *Cereal Foods World*, **46(3)**, 112-126.

Adebowale, K.O., Afolabi, T.A. & Lawal, O.S. (2002). Isolation, chemical modification and physicochemical characterisation of bambarra groundnut (*Voandzeia subterranean*) starch and flour. *Food Chemistry*, **78**, 305-311.

Adebowale, K.O. & Lawal, O.S. (2004). Comparative study of the functional properties of bambarra groundnut (*Voandzeia subterranean*), jack bean (*Canavalia ensiformis*) and mucuna bean (*Mucuna pruriens*) flours. *Food Research International*, **37**, 355-365.

Afolabi, T.A. (2012). Synthesis and physicochemical properties of carboxymethylated bambara groundnut (*Voandzeia subterranean*) starch. *International Journal of Food Science and Technology*, **47**, 445-451.

BeMiller, J.N. (2003). Carbohydrate analysis. In: *Food Analysis Third Edition* (edited by S.S. Nielsen). Pp. 145, 156. New York: Kluwer Academic/Plenum Publishers.

Brough, S.H., Azam-Ali, S.N. & Taylor, A.J. (1993). The potential of bambara groundnut (*Vigna subterranea*) in vegetable milk production and basic protein functionality systems. *Food Chemistry*, **47**, 277-283.

De Kock, C. (2013). Bambara groundnut. [WWW Document]: URL http://www.underutilized-species.org/documents/Publications/bambara_groundnut_paper.pdf. Accessed 3 September 2013.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*, **124**, 411-421.

Eltayeb, A.R.S.M., Ali, A.O., Abou-Arab, A.A. & Abu-Salem, F.M. (2011). Chemical composition and functional properties of flour and protein isolate extracted from bambara groundnut (*Vigna subterranea* (L.) Verdc.). *African Journal of Food Science*, **5(2)**, 82-90.

Gabriel, E.G., Jideani, V.A. & Ikhu-Omoregbe, D.I.O. (2013). Investigation of the emulsifying properties of Bambara groundnut flour and starch. *International Journal of Agricultural, Biosystems Science and Engineering*, **7(11)**, 508-516.

Lawal, O.S., Adebowale, K.O. & Adebowale, Y.A. (2007). Functional properties of native and chemically modified protein concentrates from bambara groundnut. *Food Research International*, **40**, 1003-1011.

Meuser, F. (2001). Technological aspects of dietary fibre. In: *Advanced Dietary Fibre Technology* (edited by B.V. McCleary & L. Prosky). Pp. 253, 262-265. UK: Blackwell Science Ltd.

Nakamura, A., Yoshida, R., Maeda, H. & Corredig, M. (2006). Soy soluble polysaccharide stabilisation at oil-water interfaces. *Food Hydrocolloids*, **20**, 277-283.

Nwanna, L.C., Enujiugha, V.N., Oseni, A.O. & Nwanna, E.E. (2005). Possible effects of fungal fermentation on bambara groundnuts (*Vigna subterranea* (L.) Verdc.) as a feedstuff resource. *Journal of Food Technology*, **3(4)**, 572-575.

Sirivongpaisal, P. (2008). Structure and functional properties of starch and flour from bambara groundnut. *Songklanakarin Journal of Science and Technology*, **30(1)**, 51-56.

Tanya, A.K.N., Mbofung, C.M.F. & Keshinro, O.O. (1997). Soluble and insoluble fiber contents of some Cameroonian foodstuffs. *Plant Foods for Human Nutrition*, **51**, 199-207.

Tharanathan, R.N. & Mahadevamma, S. (2003). Grain legumes – a boon to human nutrition. *Trends in Food Science & Technology*, **14**, 507-518.

United Nations Development Programme (2012). African Human Development Report, Pp. 1, 22.

Wang, N. & Toews, R. (2011). Certain physicochemical and functional properties of fibre fractions from pulses. *Food Research International*, **44**, 2515-2523.

Yusuf, A.A., Ayedun, H. & Sanni, L.O. (2008). Chemical composition and functional properties of raw and roasted Nigerian benniseed (*Sesamum indicum*) and bambara groundnut (*Vigna subterranean*). *Food Chemistry*, **111**, 277-282.

CHAPTER TWO
LITERATURE REVIEW

2.1 Polysaccharides as Food Constituents

Food carbohydrates are classified as important food constituents for several reasons such as being a major source of energy, dietary fibre and imparting of crucial technological properties in foods (BeMiller, 2003). The main food carbohydrates can be classified into three classes based on their degree of polymerisation; namely sugars (mono- and disaccharides), oligosaccharides and polysaccharides (Anon., 1998). These categories can be further subdivided into several sub-categories. From the three main categories, polysaccharides represent the form of at least 90% of carbohydrates available in nature and are conventionally defined by the presence of ten or more monomeric residues (BeMiller, 2003; Asp, 2001).

Polysaccharides can be subdivided into starch and non-starch polysaccharides [NSP], based on their unique compositions. The starch polymers are the only polysaccharides which are digestible by humans, and are recognised to be one of the most abundant food components found in all parts of plant materials such as tubers, seeds, roots and stems (BeMiller, 2003). NSP can be defined as a group of carbohydrates which do not contain the starch characteristic α-1–4- linked glucose and they are non-digestible in the human small intestine (Englyst *et al.*, 2007; Brennan & Tiwari, 2011). This latter group of polysaccharides was the subject of this study.

2.1.1 Overview of non-starch polysaccharides

NSP are a group of complex carbohydrates which are naturally present in many plant species; primarily in whole grains, vegetables and fruits (Brennan & Tiwari, 2011; Meyer & Tungland; 2001). NSP include hydrocolloids or food gums, which are typically added to foods as ingredients and cell wall polysaccharides which occur naturally. Some of the different NSP classes are shown in Figure 2.1. Important features which determine the physicochemical properties of NSP are the differences in sugar composition and glycosidic linkages of the various NSP components (Englyst *et al.*, 2007). The classification of NSP is done on the basis of their solubility, which classifies them into insoluble and soluble fractions. However, the precise differences in their solubility are not always clear (Sasaki *et al.*, 2004). Also commonly referred to as soluble fibre and insoluble fibre, these terms indicate the role of NSP as the principal components of dietary fibre; which also explains some of their main physiological characteristics (Brennan & Tiwari, 2011). To gain a better understanding of the physiological and technological characteristics of NSP, and their consequent importance in

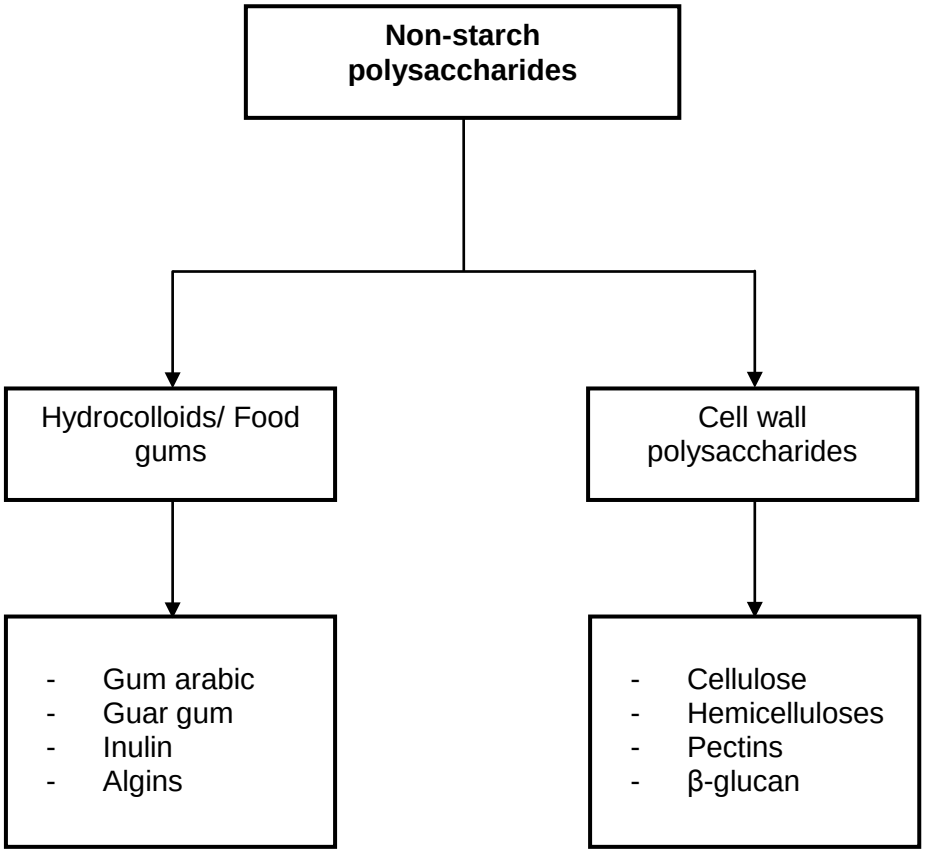


Figure 2.1 Some components of the non-starch polysaccharides (Adapted from BeMiller, 2003).

the human diet, the next sections will focus on a broad overview of dietary fibre and the different fibre fractions.

2.2 Dietary Fibre in Food and Nutrition

2.2.1 History and definition of dietary fibre

The term “dietary fibre” which first appeared in 1953 was coined by Hipsley (1953) as the non-digestible constituents that make up the plant cell wall. This term was adopted by Trowell and others (AACC, 2001) with Trowell (1972) attempting the first dietary fibre definition as “the remnants of the plant cell wall that are not hydrolysed by the alimentary enzymes of man”. Since the late 1970’s there has been consensus that “dietary fibre consists of the remnants of edible plant cells, polysaccharides, lignin and associated substances resistant to (hydrolysis) digestion by the alimentary enzymes of humans” (AACC, 2001). Since the early definitions of dietary fibre, many more have been proposed which are reviewed elsewhere (AACC, 2001; Champ *et al.*, 2003; De Vries, 2003; Dhingra *et al.*, 2012).

Some of the later definitions of dietary fibre include the AACC (2001) definition that “dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation”. The Codex Alimentarius Commission (2008, 2009) definition which is considered the most widely accepted definition of dietary fibre, states that “dietary fibre means carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to” three categories. These categories are the naturally occurring carbohydrate polymers, polymers obtained from food by physical, enzymatic or chemical means, or synthetic carbohydrate polymers; all of which have been shown to have physiological beneficial effect to health. The Codex definition also makes provision for inclusion of carbohydrate polymers with a DP of 3 – 9, and several regions have also adjusted their definition of dietary fibre accordingly. The adoption of the Codex definition and resulting approved methods is then also recommended, as it reflects on current dietary fibre research, provides continuity in food composition tables and nutrition labelling, and it facilitates comparable nutrition research amongst others (Jones, 2014).

Dietary fibre classification and composition

The complex chemical nature of dietary fibre allows for numerous ways of classification. Dietary fibre can be classified on the basis of their structure (linear or nonlinear molecular structure), properties, applications, source (animal polysaccharide, plant polysaccharide, and polysaccharides from synthetic sources) and physiological classification (Chawla & Patil, 2010; Dhingra *et al*., 2012). The conventional and most widely accepted classification of dietary fibre however, is done on the basis of their solubility in water and/or a buffer at defined pH, and/or on the basis of their fermentability by using a representative aqueous enzyme solution of the human alimentary enzymes in an in-vitro system (Chawla & Patil, 2010; Dhingra *et al.*, 2012). Based on their solubility, dietary fibre can be classified as soluble or insoluble fibres (Elleuch *et al.*, 2011) as illustrated in Figure 2.2.

Insoluble dietary fibres [IDF] are not soluble in water and include cellulose, hemicelluloses and lignin. Cellulose which is the major cell wall component in plants is composed of unbranched polymers containing thousands of glucose residues which are joined by β-1, 4-linkages. These linkages make cellulose resistant to the enzymes of the human gastrointestinal system, and when compared with the α-1, 4-linkages of starch the β-1, 4-linkages allow for greater inter- and intra-polymer hydrogen bonding (Dhingra *et al.*, 2012; Mongeau & Brooks, 2003a). The rigidity and strength required in primary and secondary plant cell walls are provided by cellulose microfibrils, which are formed by hydrogen bonding between parallel polymers (BeMiller, 2003). As cellulose-binding polysaccharides, hemicelluloses form a network together with cellulose (Brennan & Tiwari, 2011). These cell wall polysaccharides contain backbones of β-1, 4-linkages of glucose, xylose and mannose residues that are able to form extensive hydrogen bonds with cellulose (Dhingra *et al.*, 2012; Brennan & Tiwari, 2011). Hemicelluloses also represent a major fraction of dietary fibre (Mongeau & Brooks, 2003a). Lignin, formed by the condensation of aromatic alcohols, can be defined as a complex group of phenyl-propane polymers. This high-molecular weight aromatic polymer is the only substance classified as a non-carbohydrate type of dietary fibre, and due to its role in slowing dietary fibre fermentation it is of special interest (Mongeau & Brooks, 2003a; Chawla & Patil, 2010). Lignin is also very inert due to strong intramolecular bonding, and as such shows greater resistance compared to any other naturally occurring polymer (Dhingra *et al.*, 2012).

Soluble dietary fibres [SDF] are soluble in water and include pectic substances, mucilages and gums. Pectins are a complex and heterogeneous group of polysaccharides, acting as intercellular cementing substances and structural components of plant cell walls. A principal constituent of these polysaccharides is D-galacturonic acid and they are composed of homogalacturonan and rhamnogalacturonan which are covalently linked domains with distinct structures (Dhingra *et al.*, 2012; Tosh & Yada, 2010). The gelling properties of

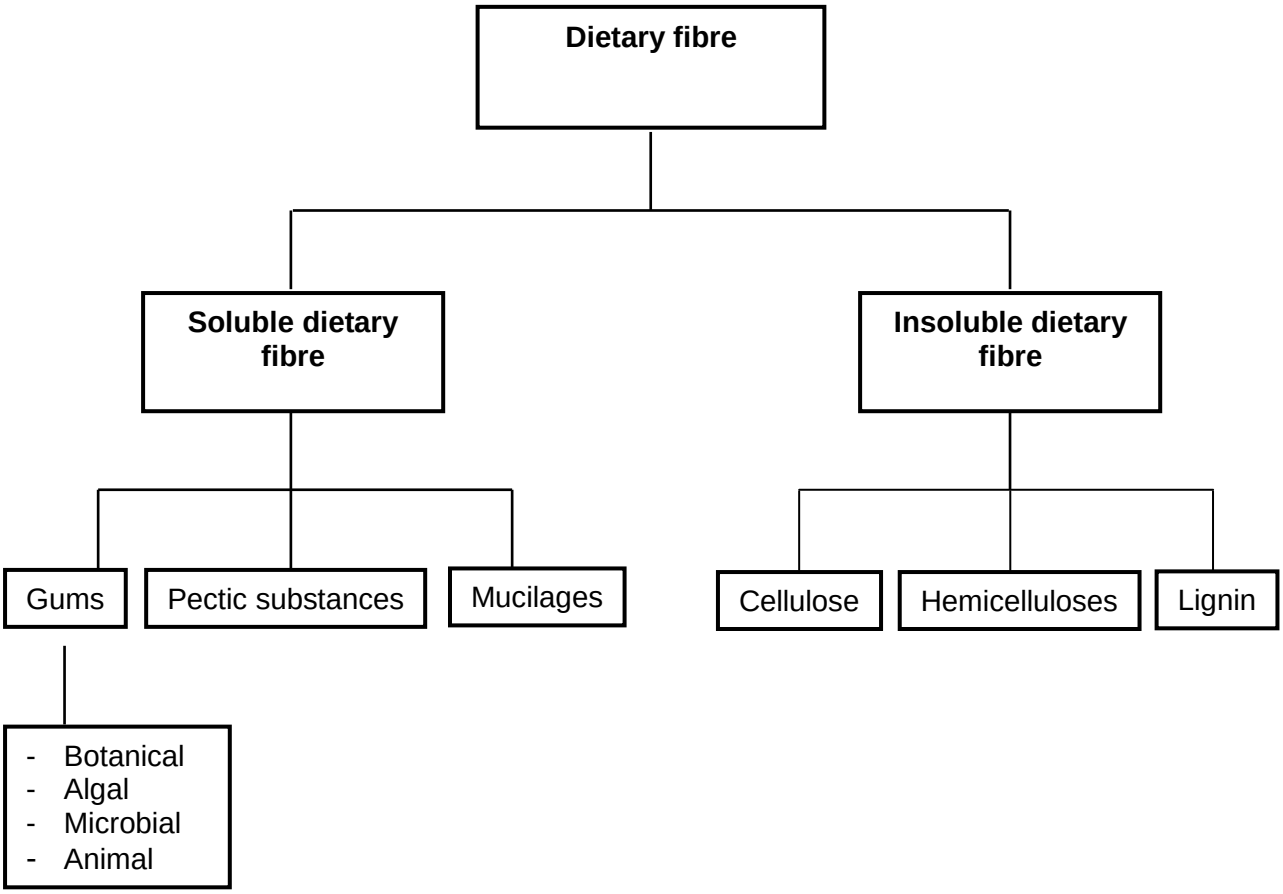


Figure 2.2 Dietary fibre classification based on solubility.

pectins are affected by their degree of esterification (Chawla & Patil, 2010). Gums and mucilages can be described as highly branched polysaccharides which perform several technological functions (Dhingra *et al.*, 2012). Gums are included in food products to perform several functions such as thickening, gelling and emulsions stabilisation. Commercially important gums can be classified into botanical (methyl cellulose, gum arabic, guar gum), algal (agar, carrageenan), microbial (xanthan gum, gellan gum) and animal (gelatin, chitosan) gums. Gums chosen for food applications are influenced by the functional characteristics required as well as the price and security of supply (Williams & Phillips, 2003). Mucilages are usually sticky substances secreted into the endosperm of plant seeds, where they are involved in the prevention of excessive dehydration (Dhingra *et al.*, 2012).

It is thus shown that insoluble and soluble dietary fibres are characterised by different compositions, each with unique functionality.

2.2.3 Dietary fibre determination in foods

As with the lack of a unique definition of dietary fibre, the measurement of dietary fibres is considerably a complex issue; consequently numerous methods for the determination of dietary fibre has been developed (Rodríguez *et al.*, 2006; Elleuch *et al.*, 2011).

The first standardised method for measurement of dietary fibre was the crude fibre method from Weende. Initially applied for fibre analysis of ruminant feeds and silage, this highly empirical method consisted of sequential extractions with hot diluted acid and alkali solutions. The crude fibre method has a recovering rate of 15% total hemicelluloses, 10 – 50% for lignin and 50 – 100% cellulose. Crude fibre is therefore not significant in human nutrition, due to the method variability and loss of dietary fibre fractions (Rodríguez *et al.*, 2006; Mongeau & Brooks, 2003b). The acid detergent fibre (ADF) and neutral detergent fibre (NDF) methods were introduced by Van Soest, by utilising anionic and cationic detergents to solubilise fats, soluble starch, simple sugars and nitrogen related compounds, without altering lignin. The fraction obtained with the ADF method consists of lignin and cellulose, whilst the weight difference between the ADF and NDF extracts represents an estimation of insoluble hemicelluloses (Rodríguez *et al.*, 2006; Mongeau & Brooks, 2003b). This group of methods can be classified as non-enzymatic-gravimetric methods, and since soluble and insoluble dietary fibre is not quantified with these methods, they are not appropriate for use in food analysis (Champ *et al.*, 2003). Following these initial methods, numerous different analytical methods have been developed for the determination of dietary fibre as are described elsewhere (Champ *et al.*, 2003; Mongeau & Brooks, 2003b; Rodríguez *et al.*, 2006; Dhingra *et al.*, 2012).

With the recent developments in dietary fibre methodology, two broad categories have been adopted for measurement of dietary fibres; namely enzymatic-gravimetric

methods and enzymatic-chemical methods (Rodríguez *et al.*, 2006; Dhingra *et al.*, 2012; Elleuch *et al.*, 2011).

Measured by the enzymatic-gravimetric methods are the indigestible polysaccharides, some resistant starch, lignin and associated compounds such as phenolic compounds and Maillard reaction products (Englyst *et al.*, 2007; Elleuch *et al.*, 2011). The key analytical principles involved include the enzymatic treatments for removal of starch and protein, followed by precipitation of the residue in 80% ethanol and isolation by filtration, total residue weight is recorded and the protein and ash contents corrected in the residue (Dhingra *et al.*, 2012; Englyst *et al.*, 2007). The enzymatic-chemical methods measure the non-starch polysaccharides and Klason lignin to obtain a quantity for total dietary fibre (Elleuch *et al.*, 2011). The analytical principles involved are the enzymatic removal of starch and its hydrolysis. The residue is precipitated with 80% ethanol and isolated by centrifuging. The non-starch polysaccharides are hydrolysed and measured as constituent sugars by chromatography or colorimetry (Englyst *et al.*, 2007; Dhingra *et al.*, 2012). The most commonly used methods to measure dietary fibre in foods is the AOAC gravimetric-enzymatic method and the Englyst enzymatic-chemical method; in comparison the AOAC method measures the highest fibre content and the Englyst method the lowest (Goñi *et al.*, 2009; Elleuch *et al.*, 2011). Garcimartin *et al.* (1995) also concluded that the AOAC method is easier and faster to perform, and that dietary fibre is not overestimated as compared to the Englyst method; which they found to be time consuming and laborious.

As investigated by several authors (Mañas *et al.*, 1994; Turowski *et al.*, 2007; Goñi *et al.*, 2009) however, these commonly used procedures may both be affected by errors and as such are not accurate enough for either scientific or commercial purposes; improvements to these methods have been recommended. Mañas *et al.* (1994) suggested centrifugation of samples after enzymatic treatments to obtain IDF residues and supernatants, followed by dialysis of supernatants to obtain the SDF fractions. Both fractions (SDF and IDF) can then be chemically quantified after acid-hydrolyses. Dialysis of supernatants were found to be more reliable than ethanol precipitation, as incomplete precipitation of SDF fractions and co-precipitation of non-fibre components have been observed with the ethanolic precipitation method. Polysaccharides such as modified cellulose fibres are also not detected by the existing methods, due to the solvent and temperature incompatibility of these methods (Turowski *et al.*, 2007). Goñi *et al.* (2009) investigated the measurement of polyphenols and resistant protein as dietary fibre constituents. A substantial amount of dietary polyphenols was found to be associated with both IDF and SDF, whereas a substantial amount of resistant protein was found in the IDF fraction. These findings support the inclusion of both polyphenols and resistant protein as dietary fibre constituents. The methodology used in this study was therefore based on these recommended improvements.

2.2.4 Physiological and functional benefits of dietary fibre

The increased intake of dietary fibre proved to have beneficial effects on health, imparting distinct physiological and functional properties when incorporated into foods (Dhingra *et al.*, 2012; Elleuch *et al.*, 2011). As a consequence of its beneficial effects, dietary fibre is considered to be amongst the important ingredients to be included in the diet (Schaafsma, 2004). The increased consumption of dietary fibre is known to have a positive effect on health by being associated with the decreasing risk of the development of several diseases (Anderson *et al.*, 2009). These diseases which can be prevented or treated with the consumption of a high fibre diet and which are of significance to public health include amongst others diabetes, cardiovascular diseases, obesity, constipation and some forms of cancer (Marlett *et al.*, 2002; Champ *et al.*, 2003; Tharanathan & Mahadevamma, 2003; Anderson *et al.*, 2009; Mann & Cummings, 2009).

Being a vital part of a healthy diet, dietary fibre has an impact on all aspects of gastrointestinal physiology; most of which are being influenced by the physicochemical characteristics of the different fibre types (Brownlee, 2011). These characteristics include the fermentability, bulk, viscosity, water holding capacity and bile acid binding of dietary fibres (Schneeman, 1998; Blackwood *et al.*, 2000; Chaplin, 2003); all of which influence the small and large intestine differently. The fermentation characteristics of dietary fibre mainly affect the large intestine, where it is responsible for increasing the microbial mass, alleviating of colon carcinogenesis and production of short chain fatty acids (Schneeman, 1998; Tharanathan & Mahadevamma, 2003). The viscosity of dietary fibre is known to slow gastric emptying and absorption rates in the small intestine (Mongeau & Brooks, 2003a). The bulk and water holding capacity of dietary fibres affect both the small and large intestinal functions. In the small intestine the bulk phase and the volume of intestinal contents is increased, leading to altered transit time, slower digestion and increased nutrient absorption. In the large intestine the bulk is increased which aids laxation, and an aqueous phase is provided for penetration of microflora leading to an increase in microbial breakdown of the polysaccharide structure (Schneeman, 1998; Tharanathan & Mahadevamma, 2003). On the basis of their physicochemical properties, distinction can be made between the effects soluble and insoluble dietary fibres have on the intestinal tract. In the small intestine, soluble dietary fibres are noted for the lowering of blood cholesterol and the regulation of blood glucose levels; while insoluble dietary fibres affect the large intestine by supporting the growth of intestinal microflora (Blackwood *et al.*, 2000; Tosh & Yada, 2010).

Apart from their influence on physiological functionality, the physicochemical properties of dietary fibres are of importance for their technological effects when incorporated into food products. Some of the important properties include fibre dimensions, rheological properties, hydration, texturising and stabilising properties, gel-forming capacity, oil-holding

capacity as well as flavour and colour (Guillon & Champ; 2000; Elleuch *et al.*, 2011). Some of these physicochemical properties and their resulting effects in different foods are further addressed in section 2.2.5.

The beneficial effects of dietary fibre in health, physiology and the distinct functional properties imparted in food products, makes dietary fibre important in the development of highly demanded functional and value-added foods (Abdul-Hamid & Luan, 2000). As such, the market for products and ingredients rich in dietary fibre has grown largely and a trend to obtain new dietary fibre food sources for use in the food industry has been observed (Chau & Huang, 2003).

Important food sources of dietary fibre include cereals and grains, legumes, fruits, vegetables and seeds (Chawla & Patil, 2010). Most of these fibre fractions are however only suitable for a limited number of applications, as a consequence of the lack of a neutral flavour and having distinctive colour. In contrast, several legume fibres have been identified for being particularly neutral in flavour as well as having beneficial physicochemical properties (Meuser, 2001). Despite these observations, the legume fibres are still being underutilised. This knowledge thus prompts the investigation of legumes as dietary fibre sources, which could be a means of highlighting the importance of this underutilised food group.

2.2.5 Food applications of dietary fibre fractions

As a consequence of the health effects and technological properties exerted by dietary fibres and specifically legume fibres, many researchers and food processors are making use of dietary fibres as new functional ingredients in several food products (Tiwari & Cummins, 2011). The high demand of value-added and functional foods[1] also allows for the addition of dietary fibres to a wide range of food products (Abdul-Hamid & Luan, 2000; Dhingra *et al.*, 2012). As reviewed by Tosh & Yada (2010), the investigated or proposed food application studies for pulse fibre fractions generally fall into the categories of fibre enrichment, nutrient fortification, fat binding and retention or texture modification. The numerous food products falling into these categories include bakery products, beverages, meat products, fish products, confectionary, frozen dairy and convenience products, dairy products, pastas, fruit preparations and soups (Endress & Fischer, 2001; Elleuch *et al.*, 2011). The technological effects exerted by fibres in some of these food groups are detailed in Table 2.1. Among

[1] Functional foods have been described by several definitions, but basically the definitions of functional foods can be classified under two approaches; simplistic definitions or complex definitions. Bech-Larsen & Grunert (2003) gave examples of simple and complex definitions of functional foods: “Foods that may provide health benefits beyond basic nutrition” and more complex “Food similar in appearance to conventional food that is intended to be consumed as part of a normal diet, but has been modified to subserve physiological roles beyond the provision of simple nutrient requirement”.

Table 2.1 Dietary fibre enrichment of selected foods[1]

Food	Type of fibre	Technological effect	Reference
Bakery products			
Bread and cookies	Mango dietary fibre	Anti-radical efficiency	Vergara-Valencia *et al.* (2007)
Cookies	Apple and lemon fibre	Lower phytic acid contents	Bilgiçli *et al.* (2007)
Bread	Carob and pea fibre	Softer crumbs	Wang *et al.* (2002)
Cookies	Sugarbeet fibre	Increased total dietary fibre	Ozturk *et al.* (2008)
Wire-cut cookies	Lemon fibre	Increased hardness	Uysal *et al.* (2007)
Flakes	Coconut fibre	Increased total dietary fibre	Trinidad *et al.* (2006)
Biscuits	Mango fibre	Improved antioxidant properties	Ajila *et al.* (2008)
Cake	Nopal fibre	Increased overall acceptability	Ayadi *et al.* (2009)
Bread	Lupin kernel fibre	Beneficial effects on blood glucose and insulin measures	Johnson *et al.* (2003)
Bread	Wheat fibre	Lower postprandial glucose concentrations	Feldheim & Wisker (2002)
Meat products			
Meat batters	Rice bran fibre	Regular fat control	Choi *et al.* (2009)
Beef frankfurters	Sugarbeet fibre	Increased total dietary fibre and water-holding capacity	Vural *et al.* (2004)
Meatballs	Oat bran	High acceptability	Yılmaz & Daglıoglu (2003)
Cooked-meat sausage	Orange fibre	Hypocaloric product	García *et al.* (2007)
Dry-cured sausage	Orange fibre	Reduction in residual nitrite levels	Fernández-Ginés *et al.* (2004)
Bologna sausage	Orange fibre	Increased antioxidant activity	Viuda-Martos *et al.* (2008)
Dry-cured sausage	Orange fibre	Enhanced organoleptic characteristics	García *et al.* (2002)
Bologna sausage	Lemon albedo	Reduction in residual nitrite levels	Fernández-Ginés *et al.* (2004)
Breakfast sausage	Citrus fibre	Increased antioxidant activity	Alesón-Carbonell *et al.* (2005)

[1] Source: Viuda-Martos *et al.* (2010).

Table 2.1 Dietary fibre enrichment of selected foods (continued)[1]

Food	Type of fibre	Technological effect	Reference
Dairy products			
Fermented milk	Citrus fibre	Increased textural properties	Sendra *et al.* (2008)
Yoghurt	Wheat and apple fibre	Decreased availabilities of both calcium and glucose	Rodríguez *et al.* (2008)
Petit-suisse cheese	Inulin	Improved sensory quality	Cardarelli *et al.* (2008)
White-brined cheese	Oat fibre	Increased textural properties	Volikakis *et al.* (2004)
Yoghurt	Asparagus fibre	Increased sensory acceptance	Sanz *et al.* (2008)
Fermented milk	Chicory inulin	Increased viability of bifidobacteria	Varga *et al.* (2006)
Yoghurt cheese	Inulin	Increased survival of probiotic bacteria	Salem *et al.* (2007)
Yoghurt	Wheat and apple fibre	Increased sensory acceptance	Staffolo *et al.* (2004)
Fish products			
Restructured hake products	Chicory fibre	Increased hardness	Cardoso *et al.* (2007)
Restructured fish products	White grape fibre	Increased antioxidant activity	Sánchez-Alonso *et al.* (2008)
Surimi	Chitosan	Increased breaking force and deformation of gels	Benjakul *et al.* (2001)
Tuna “pate”	Citrus fibre	Increased antioxidant activity	Sánchez-Zapata & Pérez-Álvarez (2008); Sánchez-Zapata *et al.* (2008)
Cod sausage	Chitosan	Increased elasticity	López-Caballero *et al.* (2005)
Fish sausage	Pea fibre	Lower fat content	Cardoso *et al.* (2008)
Restructured fish products	Wheat fibre	Increased water-holding capacity	Sánchez-Alonso *et al.* (2007)

[1] Source: Viuda-Martos *et al.* (2010).

these foods the effects of dietary fibre enrichment on bakery products and beverages have been well-documented (Rodríguez *et al.*, 2006; Elleuch *et al.*, 2011).

Dietary fibres are most commonly added to bakery products due to the ability of fibres to retain water, which prolongs freshness and consequently reduce economic losses (Elleuch *et al.*, 2011). The main food source of dietary fibre intake is bread, with commonly used wheat flour supplying a final dietary fibre content of 3 g/100 g to bread (Endress & Fischer, 2001). White bread which is the preferred choice of bread for many consumers can be used as a medium to add amounts of 5 – 6% dietary fibre isolate, to make the bread physiologically comparable with wholegrain bread (Endress & Fischer, 2001; Dean *et al.*, 2008). The benefits of fibre-enriched white bread include amongst others an enhanced bread yield, reduced bakery losses and improved bread crumb. In studies by Gómez *et al.* (2003) and Dalgetty & Baik (2006), breads fortified with commonly investigated pulse fibre fractions (peas, chickpeas and lentils) resulted in breads with acceptable quality. Fortification of bread with dietary fibre is thus not only economically viable, but could also contribute positively to human health whilst remaining sensorially acceptable to consumers.

The addition of dietary fibres to beverages leads to increased stability and viscosity; with soluble fibres being preferred above insoluble dietary fibres due to their higher dispersibility in water (Viuda-Martos *et al.*, 2010). The word beverage can be defined as a generic term which is used to classify many liquid foods as shown by the classification in Figure 2.3 (Gordon & Kubomura, 2003). Beverages (carbonated or non-carbonated) are firstly prepared as an emulsion concentrate after which they are diluted in sugar solution in order to be produced as a finished beverage (Buffo *et al.*, 2001). Beverage emulsions are considered as a unique class of oil-in-water emulsions as their consumption should be in a highly diluted form and not in the originally concentrated form (Buffo & Reineccius, 2002). Beverage emulsions are used in soft drinks to provide colour, flavour, cloudiness or a cloudy appearance. The most popular soft drink flavours are the citrus flavours which are produced from essential oils extracted from the peels of these fruits. These oils are not water-soluble and are consequently incorporated in the form of a beverage emulsion (Buffo *et al.*, 2001). Beverage emulsions must be stable under concentrated and highly diluted conditions. Polysaccharides/hydrocolloids are typically used as stabilisers and emulsifiers in beverage emulsions. The water-soluble polysaccharide, gum arabic, is the most commonly and best-known polysaccharide used to stabilise beverage emulsions. Gum arabic is widely used and the cost of this polysaccharide is fairly high due to its unstable supply. A need has thus been identified for sourcing of other hydrocolloids to be used in beverage emulsions (Nakamura *et al.*, 2004; Buffo *et al.*, 2001). As described by Tan (2004), hydrocolloids suitable for use as stabilisers must have a low viscosity in solution, high solubility in cold water, no thickening/gelling effects with aging and high emulsifying capacity. Soluble dietary fibres

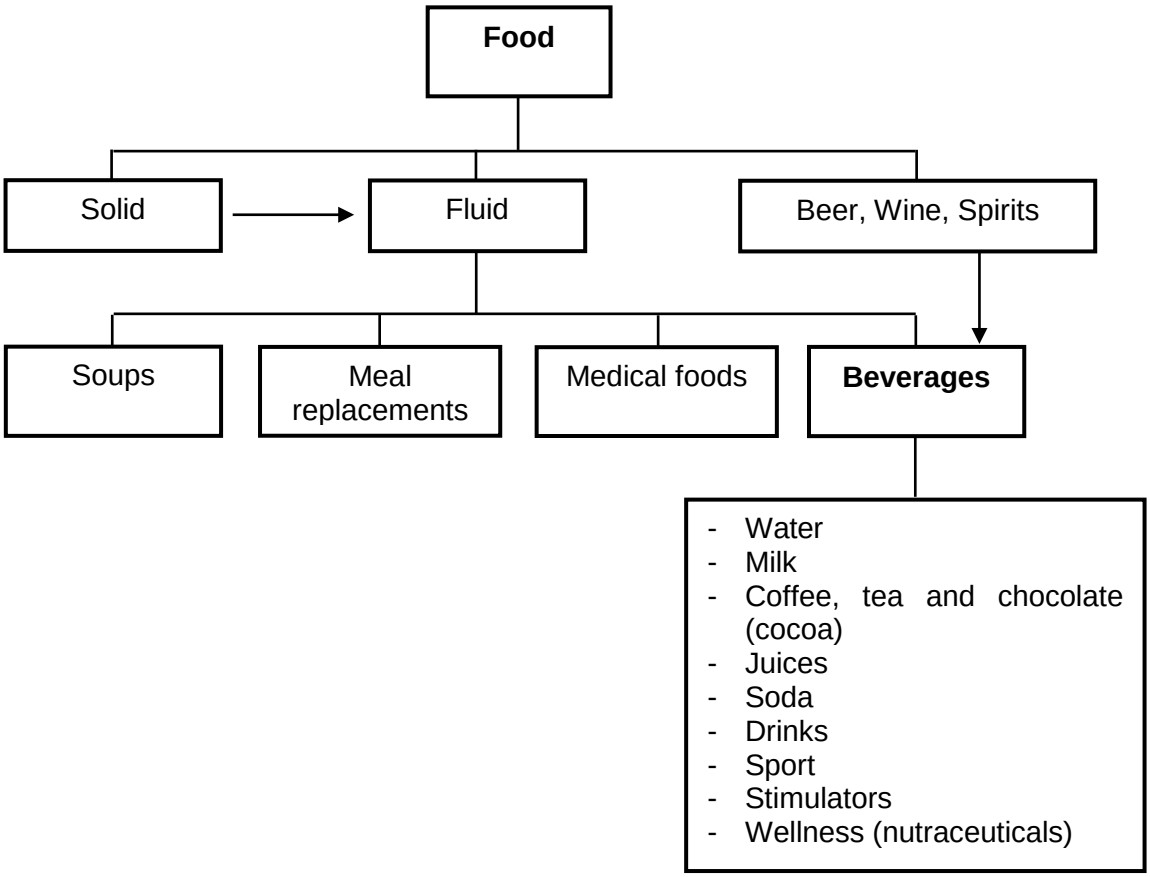


Figure 2.3 Beverage categories (Adapted from Gordon & Kubomura, 2003).

have a higher dispersibility in water, exhibits a greater ability to provide viscosity, form gels and to act as emulsifiers as compared to the insoluble fibre fractions. For this reason, soluble dietary fibres are added to beverages and drinks to increase their viscosity and stability (Rodríguez *et al.*, 2006; Elleuch *et al.*, 2011). Tosh & Yada (2010) also noted that the low viscosity of soluble pulse fibres would make them suitable supplements for the enrichment of non-viscous products such as functional beverages or juices. A legume fibre fraction which has been explored and suggested as emulsifier for beverage emulsions is the soybean soluble polysaccharide (SSPS), a fraction which is extracted from soybean cotyledons. SSPS provides stable emulsions and exhibits good emulsifying properties at different pH and ionic concentrations (Nakamura *et al.*, 2004; Nakamura *et al.*, 2006; Nakauma *et al.*, 2008). These results are evident that legume/pulse fibres can be successfully incorporated into beverage emulsions, thus creating a need for evaluation of other legume fibres.

2.3 Legumes in Human Nutrition

2.3.1 Characterisation and nutritional composition of legumes

Food legumes, species of the plant family Leguminosae, consist of 650 genera and more than 18000 different species. They are ranked second after cereal grains as the most important food source in the world (Tiwari *et al.*, 2011). Legumes can be categorised into leguminous oilseeds which are legume seeds that contain fat in large quantities (such as peanuts and soybeans) or pulses which refer to legume seeds containing small amounts of fat (such as common beans and peas). The term pulses are however more commonly used when referring to legumes (Sathe & Deshpande, 2003; Dalgetty *et al.*, 2003). Mature legume seeds have three major components namely the cotyledons (comprising 90% of total seed weight), seed coat (8%) and the embryo (2%) (Dalgetty *et al.*, 2003) as illustrated in Figure 2.4.

Known for their health-promoting and nutritional benefits, the investigation of pulses as an abundant, nutrient-rich and underutilised food source has been on the increase (Derbyshire, 2011). As shown in Table 2.2, eleven primary pulses are recognised by the Food and Agriculture Organisation [FAO]. The varied nutritional profile of pulses makes them unique, in the sense that it is difficult to compare them to the nutritional profiles from other food sources. Pulses are rich in proteins and are good sources of slow release carbohydrates (such as dietary fibre), minerals and vitamins. In comparison with other foods they have a low fat, sodium and energy content, which makes them a fairly nutrient dense

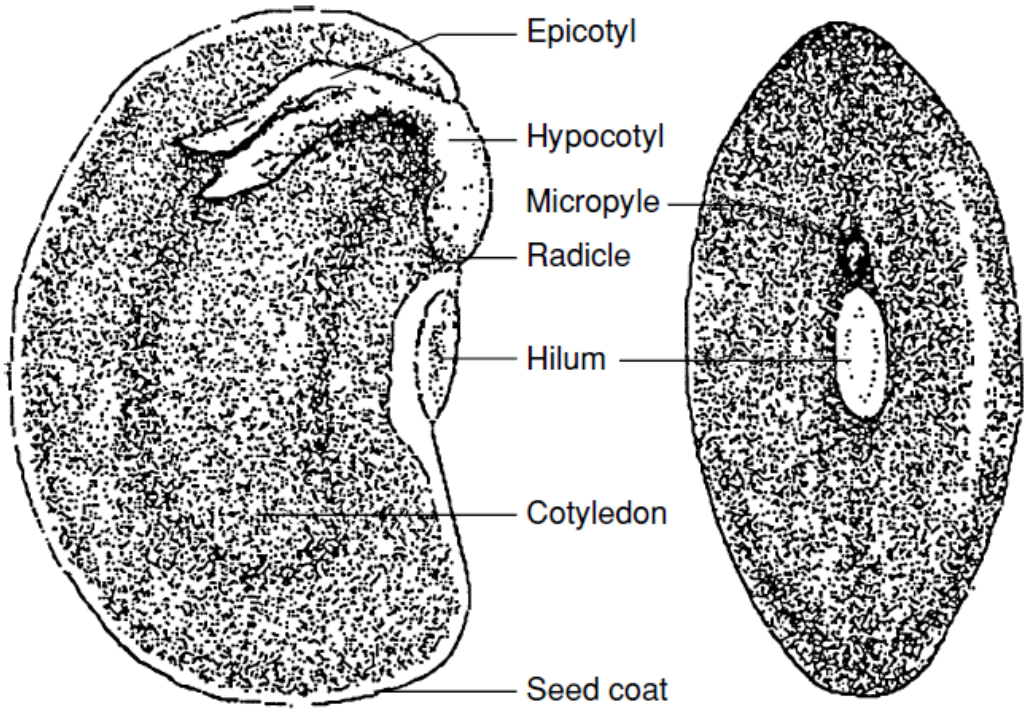


Figure 2.4 Anatomical structure of a legume seed (Source: Uebersax & Occeña, 2003).

Table 2.2 Commonly consumed pulses[1]

Pulse class	Common/ local names	Botanical name
1. Dry beans		*Phaseolus* spp., *Vigna* spp.
	Kidney bean, haricot bean, pinto bean, navy bean	*Phaseolus vulgaris*
	Lima bean, butter bean	*Phaseolus lunatus*
	Azuki bean, adzuki bean	*Vigna angularis*
	Mung bean, golden gram, green gram	*Vigna radiate*
	Black gram, urad	*Vigna mungo*
	Scarlet runner bean	*Phaseolus coccineus*
	Rice bean	*Vigna umbellate*
	Moth bean	*Vigna acontifolia*
	Tepary bean	*Phaseolus acutifolius*
2. Dry broad beans		*Vicia faba*
	Horse bean	*Vicia faba equine*
	Broad bean	*Vicia faba*
	Field bean	*Vicia faba*
3. Dry peas		*Pisum* spp.
	Garden pea	*Pisum sativum* var. *Sativum*
	Protein pea	*Pisum sativum* var. *Arvense*
4. Chickpea	Garbanzo, Bengal gram	*Cicer arietinum*
5. Dry cowpea	Black-eyed pea, black-eye bean	*Vigna unguiculata*
6. Pigeon pea	Arhar/ Toor, cajan pea, Congo bean	*Cajanus cajan*
7. Lentil		*Lens culinaris*
8. Bambara groundnut	Earth pea	*Vigna subterranean*
9. Vetch	Common vetch	*Vicia sativa*
10. Lupins		*Lupinus* spp.
11. Minor pulses	Lablab, hyacinth bean	*Lablab purpureus*
	Jack bean	*Canavalia ensiformis*
	Sword bean	*Canavalia gladiate*
	Winged bean	*Psophocarpus teragonolobus*
	Velvet bean, cowitch	*Mucuna pruriens* var. *Utilis*
	Yam bean	*Pachyrrizus erosus*

[1] Source: Tiwari *et al.* (2011).

food source (Derbyshire, 2011; Tharanathan & Mahadevamma, 2003). Pulses are thus overall an abundant source of important nutrients.

2.3.2 Dietary fibre fractions from pulses

Pulses are traditionally consumed as whole cooked grains, whilst the processing and/or fractionation of pulses into flours and major constituents (protein concentrates and isolates, fibres and starch) are on the increase as a result of the growing trend towards ready-to-eat, convenient and value added food products, which require the use of ingredients which can be easily handled (Farooq & Boye, 2011). Pulse fibres also have superior functional properties and this leads to a great potential for their use in various food applications (Wang & Toews, 2011).

Fibre fractions which are one of the major pulse fractions can be incorporated into commercial food products to enhance fibre content and/or to act as functional ingredients. Pulse fibre fractions can be distinguished as inner and outer fibres. Inner fibres refer to dietary fibre of the cotyledon cells which consists mainly of cell wall polysaccharides; outer fibres refer to fibres derived from seed coats (hulls) and consist mainly of insoluble hemicelluloses, cellulose and varying levels of lignin (Farooq & Boye, 2011; Meuser, 2001; Pfoertner & Fischer, 2001). In comparison, hulls have higher dietary fibre content than cotyledons (Pfoertner & Fischer, 2001). Commercially available fibre fractions for use in food applications are the hull and cotyledon fibres from peas; however fibre fractions from other pulse sources are scarce (Wang & Toews, 2011). The most commonly investigated pulse fibre fractions are those from peas, lentils, chickpeas and navy beans as described by Dalgetty & Baik (2003) and Wang & Toews (2011); some functional properties of these legume fibres are shown in Table 2.3. These fibres are only from four of the eleven primary FAO recognised pulses, thus leaving other pulse classes to be investigated (Table 2.2). From this knowledge, the gap in the market would be to expand the utilisation of pulse derived fibres, which would lead to enhanced human health as well as an increase in the market value of pulse crops (Wang & Toews, 2011; Tosh & Yada, 2010). Bambara groundnut [BGN], one of the eleven underutilised primary pulses, was the subject of this study.

Table 2.3 Functional properties of some pulse fibre fractions[1]

Fibre source	Bulk density ($g.ml^{-1}$)	Swelling capacity ($ml.g^{-1}$)	Water retention capacity ($g.g^{-1}$)	Fat absorption ($g.g^{-1}$)
Hull fibres				
Pea	0.75	1.88	1.51	1.51
Lentil	0.81	2.38	1.63	1.63
Chickpea	0.73	3.61	1.76	1.76
Cotyledon fibres				
Pea	0.21	5.56 – 19.2	8.5 – 13.4	1.63 – 6.93
Lentil	0.36	8.04 – 27.1	8.9 – 11.4	1.10 – 4.01
Chickpea	0.34	4.28 – 24.6	7.7 – 10.1	1.30 – 4.25
Navy bean	na	16.0	6.8	1.06
Commercial Cotyledon fibres[2]	na	6.4 – 8.3	3.7 – 4.5	1.23 – 1.33

[1] Data sources: Dalgetty & Baik (2003), Wang & Toews (2011)
[2] Commercial cotyledon fibres as measured by Wang & Toews (2011)
na: not analysed

2.4 Bambara Groundnut

2.4.1 Origin and background of BGN

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is classified under the family Fabaceae (also known as the Leguminosae), sub-family Faboidea and genus *Vigna* (Bamishaiye *et al.*, 2011). BGN is an easy-to-cultivate legume seed which is widely grown throughout tropical Africa, Indonesia, Malaysia and Sri Lanka (Eltayeb *et al.*, 2011). In a study by Azam-Ali *et al.* (2001) the utilisation of computer-based analytical tools allowed for prediction of potential regions for BGN cultivation and apart from large areas in Africa, land areas in some parts of Australia and the Mediterranean basin of Europe were also predicted as suitable areas.

BGN originated in West Africa with its name derived from the Bambara tribe who now lives in Mali (Nwanna *et al.*, 2005). In some parts of Africa BGN is known by various synonyms such as Okpa [Nigeria], jugo beans [South Africa], Gurjiya [Nigeria] and Nyimo beans [Zimbabwe] (Bamishaiye *et al.*, 2011). In South Africa BGN is found in Mpumalanga, Limpopo and KwaZulu-Natal. It was introduced into South Africa by either one of two tribes. The Bolebedu of Letaba tribe who arrived south of Limpopo (before the Venda tribe) claims that they came with BGN from the north. The Venda tribe however claims that they were the first to bring BGN from Central Africa to the then Transvaal and this claim is supported by two factors; namely the Venda name Nduhu-mvenda which means the groundnut of Vendaland and the harvest ritual used among the Venda which is normal for phonda (*Vigna subterranea*) (Swanevelder, 1998).

BGN is a crop with great potential to sustain the dietary needs of both urban and rural communities (Jideani & Diedericks, 2014), but due to several negative connotations associated with the crop, it is being given less priority in land allocation and lesser value (Bamishaiye *et al.*, 2011). Thus, despite its several important attributes BGN yields are still low and its full economic significance remains to be determined (Mkandawire, 2007).

2.4.2 Physical characteristics of BGN plant and seeds

BGN is a leguminous, herbaceous and typical short-day plant which is grown predominantly for its underground produced seeds. The crop grows close to the ground and can be grown without the use of expensive chemicals and fertilisers which are usually difficult to obtain in isolated areas (Hillocks *et al.*, 2012). The BGN crop have many favourable characteristics including its abundance in nitrogen which contributes to soil fertility maintenance, its ability to produce yields in areas with minimal rain fall (heavy rain fall are also tolerated before the plant reaches maturity) and poor soils, and its suitability to be intercropped with other crops such as maize (Hillocks *et al.*, 2012; Mkandawire, 2007).

Propagation of BGN occurs via its seeds, which are obtained from local markets or retained from the previous harvest (Stephens, 2012). Cultivar and weather dependent, the BGN plant matures in three to six months with the flowers and pods identified to be the essential parts of the plant (Directorate Plant Production [DPP], 2011). The plant appearance is generally classified into two types; the bunch or spreading types with the former usually being self-pollinated and the latter being cross-pollinated by ants. Leaves are trifoliate and grow from the well-developed compact tap root system from the stems which are short and lateral. Flowering begins at 30 – 35 days after sowing, with the flower stem elongating after fertilisation and penetrating the soil (area of fruit development). The development of pods begins about 30 days after the fertilisation process with the seeds developing after the pods in approximately ten days. The pods are formed underground and are characterised as small being approximately 1.5 cm in length, they may be wrinkled and slightly oval or round shaped with one to two seeds. Colour of the pods varies from a yellowish-white for unripe pods to yellowish-brown or purple for mature pods (Mkandawire, 2007; Swanevelder, 1998).

The seeds of BGN are usually round, hard and smooth with varying sizes. The colour of the seeds vary from cream, white, red, dark-brown, black or a combination of these colours and it may also be speckled with or without hilum colouration (Mkandawire, 2007; Bamishaiye *et al.*, 2011). The BGN seed coats are extremely tough, which makes them resistant to weevil attack and allows for storage of the seeds for long periods without loss (Linnemann, 1990; Brough *et al.*, 1993). The physical properties of seeds and grains are important to consider in the design of process equipment or in determining their behaviour during certain processes. Mpotokwane *et al.* (2008) evaluated the physical properties of several BGN seed landraces from Botswana. The authors found that BGN seeds are irregular in size and shape, and that they will roll as opposed to slide. Illustrations of the BGN plant and different seed varieties are shown in Figure 2.5.

2.4.3 Nutritional and phytochemical characteristics of BGN

BGN seeds contain on average 63% carbohydrate, 19% protein and 6.5% fat; amounts which are regarded as sufficient to make the seed a complete food (Bamishaiye *et al.*, 2011). As shown in Table 2.4, the nutritional composition of BGN compares fairly well to more commonly utilised and commercialised legume seeds such as soybean, cowpea and chickpea. The high carbohydrate fraction of BGN mostly contains starch and non-starch polysaccharides (Bamishaiye *et al.*, 2011). It is therefore not surprising that BGN seeds also

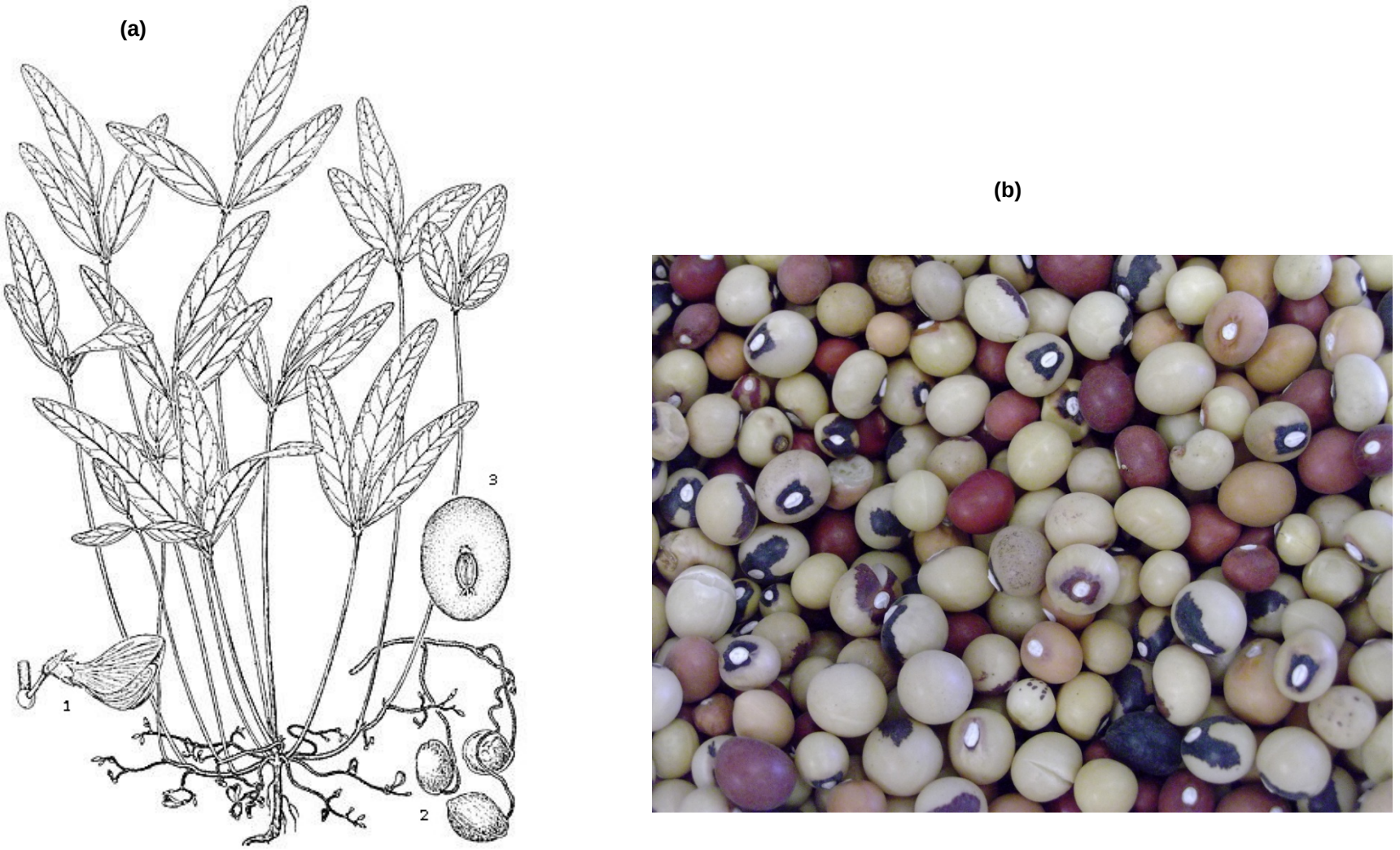


Figure 2.5 BGN **(a)** flowering plant (1: flower, 2: fruits, 3: seed) [Source: Brink *et al.* (2006)] and **(b)** various seeds.

Table 2.4 Nutritional composition of BGN and some commonly utilised legumes[1,2]

	Bambara groundnut	Soybean	Chickpea	Cowpea
Calories (kCal)	390.0	416.0	364.0	343.0
Protein (g)	20.8	36.5	19.3	23.8
Carbohydrates (g)	61.9	30.2	60.6	59.6
Fat (g)	6.6	19.9	6.0	2.1

[1] Source: De Kock (2013).
[2] BGN: Bambara groundnut.

have high dietary fibre content comparable to other legume seeds (Table 2.5), thus making them a relatively good source of these carbohydrate fractions (Tanya *et al.*, 1997). Investigation of the dietary fibre fractions from BGN is however still lacking.

BGN is a good source of iron, potassium, calcium and nitrogen, and it contains high levels of the essential sulphur-containing amino acid methionine (Mkandawire, 2007; De Kock, 2013). Nti (2009) also showed how boiling with dehulling and dehulling of BGN seeds positively impacts the protein content in BGN flours. Protein which was the highest in the undehulled (27.4%) black white-eye variety significantly [$p \leq 0.05$] increased when seeds were dehulled (28.6%) and boiled before dehulling (28.6%). Importantly, the tannins which are anti-nutritional components, were significantly [$p \leq 0.05$] decreased from 15.4 mg CE.g^{-1} in undehulled black white-eye BGN flour to 1.2 mg CE.g^{-1} in dehulled and 0.1 mg CE.g^{-1} in boiled and dehulled samples. The positive effects of processing techniques on BGN nutritional composition is noteworthy, and could allow for utilisation of BGN in the development of important food products such as weaning foods where high protein formulations are important.

The limited information available on the phytochemical components of BGN seeds such as the presence of three anthocyanins (delphinidin 3-*O*-β-glucoside, petunidin 3-*O*-β-glucoside and malvidin 3-*O*-β-glucoside) (Pale *et al.*, 1997) and the flavonoid kaempferol-3-*O*-glucoside-7-rhamnoside presence which imparts many health benefits and reduces many chronic illnesses (Chen & Chen, 2013), is noteworthy and could place BGN in a significant role contributing to human health.

2.4.4 Food and pharmaceutical uses of BGN

BGN seeds are consumed in various forms (immature and fully matured form) and in various ways. Immature seeds are more palatable, whilst the harder matured seeds are boiled or roasted to make them softer and more pleasant tasting and sweet (DPP, 2011; Stephens, 2012). Dried BGN seeds can be ground into flour to form cakes, whilst immature unshelled or shelled seeds are pounded and boiled into a stiff porridge. BGN may also be served as relishes and appetisers (Brough *et al.*, 1993; Uebersax & Occeña, 2003). Matured seeds are often consumed as porridge by mixing the ground BGN (flour) with butter or oil; or the dried seeds are boiled and combined with maize or plantains (Brink, 2006; DPP, 2011). BGN have many traditional African uses (Table 2.6) however, this crop is still plagued with underutilised status. In an attempt to change this status of BGN and highlight its potential value as sustainable food security crop, several researchers have addressed the value addition potential of BGN and specific functional properties of the BGN flour, starch and protein fractions (Brough *et al.*, 1993; Adebowale *et al.*, 2002; Adebowale & Lawal, 2004; Lawal *et al.*, 2007; Yusuf *et al.*, 2008; Eltayeb *et al.*, 2011; Jideani & Murevanhema, 2013).

Table 2.5 Dietary fibre contents of selected legume seeds[1,2]

Legume	Botanical name	IDF (g.100 g^{-1} dry weight)	SDF (g.100 g^{-1} dry weight)	TDF (g.100 g^{-1} dry weight)
Bambara groundnut	*V. subterranea*	23.0±0.6	3.5±0.4	26.5
Black kidney beans	*Ph. vulgaris*	24.7±0.7	4.4±0.3	29.1
White kidney beans	*Ph. vulgaris*	24.7±0.4	1.2±0.2	25.9
Cow peas	*Phaseolus* sp.	25.5±0.9	2.8±0.1	28.3
Nduk	*I. gabonensis*	24.1±0.3	15.9±0.3	40.0

[1] Tanya *et al* (1997)
[2] IDF: Insoluble dietary fibre, SDF: Soluble dietary fibre, TDF: Total dietary fibre.

Table 2.6 Some food uses of Bambara groundnut in parts of Africa[1]

Country	BGN food uses	Reference
Cameroon	Testa-free fresh seeds – consumed as a complete meal by cooking with seasoning, or ground to prepare a traditional pudding sometimes with addition of taro leaves	Nguy-Ntamag, 1997
Northern Ghana	Dry BGN seeds – boiled and crushed seeds used to form cakes/balls followed by frying and adding to stews; BGN is also made into a paste and used in traditional dishes 'tubani' (steamed bean paste) and 'koose'/'akla' (fried bean paste)	Doku, 1997; Nti, 2009
Southern Ghana	'Aboboi' – prepared by soaking BGN seeds overnight followed by boiling (with/without capsicum pepper and salt) to produce a type of porridge/blancmange; served with 'gari' or plantain (ripe, fried or mashed)	Doku, 1997; Nti, 2009
Kenya – Kambe & Giriama tribes	Dry BGN seeds are prepared by removal of the seed coat through pounding, winnowing and boiling the seeds until cooked; cooked seeds are pounded and mixed with coconut juice – this preparation is cooked and stirred until smooth, and served with 'ugali' or rice	Ngugi, 1997
Nigeria	Paste prepared from BGN flour used in preparation of 'moi moi' and 'akara' (bean balls); 'okpa' (steamed gel prepared by slurry of BGN)	Obizoba, 1983; Uvere *et al.*, 1999
South Africa	BGN (sometimes with peanuts) are added to millet or maize and the mixture boiled to form a stiff dough; this dough is salted and made into a ball known as 'tshidzimba' (Venda), 'sekome' (Sesotho) or 'tihove' (Shangaan)	Swanevelder, 1997

[1] Source: Jideani & Diedericks (2014).

BGN reportedly also have medicinal value as it is used in several African communities for the treatment of various ailments. DPP (2011) reported the use of a mixture of BGN and boiled maize water for treatment of diarrhoea; and chewing and swallowing of raw BGN seeds to alleviate pregnancy associated nausea. Koné *et al.* (2011) also highlighted the following medicinal uses of BGN: water from boiled BGN seeds is used as internal bruising treatment and water/ crushed seeds mixture is used as treatment for cataracts; roasted BGN seeds are recommended for treatment of polymenorrhea; BGN incorporation in diets of young rural children helps to overcome Kwashiorkor (protein deficiency); a Nigerian tribe (Igbos) use BGN seeds for venereal diseases treatment; and other ailments such as anaemia, ulcers and menorrhagia could also be treated with BGN. There is still a wide gap for detailed study on the pharmaceutical value of BGN, which could be a means of challenging the underutilised status of this crop.

2.5 Conclusion

It is evident that the inclusion of dietary fibres in food products is of great importance for several technological, economical and human health reasons. Consequently the need for new dietary fibre food sources is increasing. BGN is one such food source with a rich nutritional profile. By tapping into just one of the many nutritional "gaps" of BGN, the importance of this crop could be greatly highlighted and realised, and moreover the underutilised status of BGN and its potential as food security crop could be addressed.

References

AACC (2001). The definition of dietary fibre (Report of the Dietary Fibre Definition Committee to the Board of Directors of the AACC). *Cereal Foods World*, **46(3)**, 112-126.

Abdul-Hamid, A. & Luan, Y.S. (2000). Functional properties of dietary fibre prepared from defatted rice bran. *Food Chemistry*, **68**, 15-19.

Adebowale, K.O., Afolabi, T.A. & Lawal, O.S. (2002). Isolation, chemical modification and physicochemical characterisation of bambarra groundnut (*Voandzeia subterranean*) starch and flour. *Food Chemistry*, **78**, 305-311.

Adebowale, K.O. & Lawal, O.S. (2004). Comparative study of the functional properties of bambarra groundnut (*Voandzeia subterranean*), jack bean (*Canavalia ensiformis*) and mucuna bean (*Mucuna pruriens*) flours. *Food Research International*, **37**, 355-365.

Ajila, C.M., Leelavathi, K. & Prasada-Rao, U.J.S. (2008). Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. *Journal of Cereal Science*, **48(2)**, 319-326.

Alesón-Carbonell, L., Fernández-López, J., Pérez-Álvarez, J.A. & Kuri, V. (2005). Functional and sensory effects of fibre-rich ingredients on breakfast fresh sausages manufacture. *Food Science and Technology International*, **11(2)**, 89-97.

Anderson, J.W., Baird, P., Davis Jr., R.H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V. & Williams, C.L. (2009). Health benefits of dietary fibre. *Nutrition Reviews*, **67(4)**, 188-205.

Anonymous (1998). FAO/WHO Report 66. Carbohydrates in human nutrition. FAO, Rome (As cited by Asp, 2001).

Asp, N. (2001). Development of dietary fibre methodology. In: *Advanced Dietary Fibre Technology* (edited by B.V. McCleary & L. Prosky). Pp. 79-80. UK: Blackwell Science Ltd.

Ayadi, M.A., Abdelmaksoud, W., Ennouri, M. & Attia, H. (2009). Cladodes from *Opuntia ficus* indica as a source of dietary fiber: effect on dough characteristics and cake making. *Industrial Crops and Products*, **30(1)**, 40-47.

Azam-Ali, S., Aguilar-Manjarrez, J. & Bannayan-Avval, M. (2001). A global mapping system for Bambara groundnut production. In: *FAO Agricultural Information Management Series*. Pp. 1-47. Rome: Food and Agriculture Organization of the United Nations.

Bamishaiye, O.M., Adegbola, J.A. & Bamishaiye, E.I. (2011). Bambara groundnut: an under-utilized nut in Africa. *Advances in Agricultural Biotechnology*, **1**, 60-72.

Bech-Larsen, T. & Grunert, K.G. (2003). The perceived healthiness of functional foods: A conjoint study of Danish, Finnish and American consumers' perception of functional foods. *Appetite*, **40**, 9-14.

BeMiller, J.N. (2003). Carbohydrate analysis. In: *Food Analysis Third Edition* (edited by S.S. Nielsen). Pp. 145, 146, 156, 163. New York: Kluwer Academic/Plenum Publishers.

Benjakul, S., Visessanguan, W., Tanaka, M., Ishizaki, S., Suthidham, R. & Sungpech, O. (2001). Effect of chitin and chitosan on gelling properties of surimi from barred garfish (*Hemiramphus far*). *Journal of the Science of Food and Agriculture*, **81(1)**, 102-108.

Bilgiçli, N., Ibanoglu, S. & Herken, E.N. (2007). Effect of dietary fibre addition on the selected nutritional properties of cookies. *Journal of Food Engineering*, **78**, 86-89.

Blackwood, A.D., Salter, J., Dettmar, P.W. & Chaplin, M.F. (2000). Dietary fibre, physicochemical properties and their relationship to health. *The Journal of The Royal Society for the Promotion of Health*, **120**, 242-247.

Brennan, C. & Tiwari, U. (2011). Functional and physicochemical properties of non-starch polysaccharides. In: *Pulse Foods: Processing, Quality and Nutraceutical Applications*

(edited by B.K. Tiwari, A. Gowen & B. McKenna). Pp. 157, 158, 160. London, UK: Elsevier Inc.

Brink, M., Ramolemana, G.M. & Sibuga, K.P. (2006). *Vigna subterranea* (L.) Verdc. In: *Plant Resources of Tropical Africa/ Ressources végétales de l'Afrique tropicale* (edited by M. Brink & G. Belay). Wageningen, Netherlands: PROTA4U.

Brough, S.H., Azam-Ali, S.N. & Taylor, A.J. (1993). The potential of bambara groundnut (*Vigna subterranea*) in vegetable milk production and basic protein functionality systems. *Food Chemistry*, **47**, 277-283.

Brownlee, I.A. (2011). The physiological roles of dietary fibre. *Food Hydrocolloids*, **25**, 238-250.

Buffo, R.A., Reineccius, G.A. & Oehlert, G.W. (2001). Factors affecting the emulsifying and rheological properties of gum acacia in beverage emulsions. *Food Hydrocolloids*, **15**, 53-66.

Buffo, R.A. & Reineccius, G.A. (2002). Modeling the rheology of concentrated beverage emulsions. *Journal of Food Engineering*, **51**, 267-272.

Cardarelli, H.R., Buriti, F.C.A., Castro, I.A. & Saad, S.M.I. (2008). Inulin and oligofructose improve sensory quality and increase the probiotic viable count in potentially symbiotic petit-suisse cheese. *LWT – Food Science and Technology*, **41(6)**, 1037-1046.

Cardoso, C., Mendes, R. & Nunes, M.L. (2007). Effect of transglutaminase and carrageenan on restructured fish products containing dietary fibres. *International Journal of Food Science and Technology*, **42(11)**, 1257-1264.

Cardoso, C., Mendes, R. & Nunes, M.L. (2008). Development of a healthy low-fat fish sausage containing dietary fibre. *Internal Journal of Food Science and Technology*, **43(2)**, 276-283.

Champ, M., Langkilde, A., Brouns, F., Kettlitz, B. & Collet, Y.L.B. (2003). Advances in dietary fibre characterisation: 1. Definition of dietary fibre, physiological relevance, health benefits and analytical aspects. *Nutrition Research Reviews*, **16**, 71-82.

Chaplin, M.F. (2003). Fibre and water binding. *Proceedings of the Nutrition Society*, **62**, 223-227.

Chau, C.F. & Huang, Y.L. (2003). Comparison of the chemical composition and physicochemical properties of different fibers prepared from peel of *Citrus sinensis* L. Cv. Liucheng. *Journal of Agricultural and Food Chemistry*, **51**, 2615-2618.

Chawla, R. & Patil, G.R. (2010). Soluble dietary fibre. *Comprehensive Reviews in Food Science & Food Safety*, **9**, 178-196.

Chen, A.Y. & Chen, Y.C. (2013). A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. *Food Chemistry*, **138(4)**, 2099-2107.

Choi, Y.S., Choi, Y.J., Han, D.J., Kim, H.Y., Lee, M.A., Kim, H.W., Jeong, J.Y. & Kim, C.J. (2009). Characteristics of low-fat meat emulsion systems with pork fat replaced by vegetable oils and rice bran fiber. *Meat Science*, **82(2)**, 266-271.

Codex Alimentarius Commission (2008). Report of the 30th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses. In: *ALINORM 09/32/26*. 3-7 November 2008. Cape Town, South Africa.

Codex Alimentarius Commission (2009). Report of the 31st Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses. In: *ALINORM 10/33/26*. 2-6 November 2009. Düsseldorf, Germany.

Dalgetty, D.D. & Baik, B. (2003). Isolation and characterization of cotyledon fibers from peas, lentils, and chickpeas. *Cereal Chemistry*, **80(3)**, 310-315.

Dalgetty, D.D. & Baik, B. (2006). Fortification of bread with hulls and cotyledon fibers isolated from peas, lentils, and chickpeas. *Cereal Chemistry*, **83(3)**, 269-274.

Dalgetty, D.D., Baik, B. & Swanson, B.G. (2003). Pulses. In: *Encyclopedia of Food Sciences and Nutrition*, 2nd Edition (edited by B. Caballero, L. Trugo & P.M. Finglas). Pp. 4880-4887. New York: Elsevier Science Ltd.

Dean, M., Raats, M.M. & Shepherd, R. (2008). Consumers and functional cereal products. In: *Technology of functional cereal products* (edited by B.R. Hamaker). Pp. 8-9. England: Woodhead Publishing Limited.

De Kock, C. (2013). Bambara groundnut. [WWW Document]: URL http://www.underutilized-species.org/documents/Publications/bambara_groundnut_paper.pdf. Accessed on 3 September 2013.

Derbyshire, E. (2011). The nutritional value of whole pulses and pulse fractions. In: *Pulse Foods: Processing, Quality and Nutraceutical Applications* (edited by B.K. Tiwari, A. Gowen & B. McKenna). Pp. 363-365. London, UK: Elsevier Inc.

De Vries, J.W. (2003). On defining dietary fibre. *Proceedings of the Nutrition Society*, **62**, 37-43.

Dhingra, D., Michael, M., Rajput, H. & Patil, R.T. (2012). Dietary fibre in foods: A review. *Journal of Food Science and Technology*, **49(3)**, 255-266.

Directorate Plant Production [DPP] (2011). Production guidelines for Bambara groundnuts. Pp. 1-10. Pretoria, South Africa: Department of Agriculture, Forestry and Fisheries.

Doku, E.V. (1997). Country Reports: Ghana. Bambara groundnut *Vigna subterranea* (L.) Verdc. Promoting the conservations and use of underutilized and neglected crops 9. In: *Proceedings of the Workshop on Conservation and Improvement of Bambara Groundnut* (*Vigna subterranea* (L.) Verdc.) (edited by J., Heller, F. Begemann & J. Mushonga). November 1995. Harare, Zimbabwe.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*, **124**, 411-421.

Eltayeb, A.R.S.M., Ali, A.O., Abou-Arab, A.A. & Abu-Salem, F.M. (2011). Chemical composition and functional properties of flour and protein isolate extracted from bambara groundnut (*Vigna subterranea* (L.) Verdc.). *African Journal of Food Science*, **5(2)**, 82-90.

Endress, H. & Fischer, J. (2001). Fibres and fibre blends for individual needs: A physiological and technological approach. In: *Advanced Dietary Fibre Technology* (edited by B.V. McCleary & L. Prosky). Pp. 290-297. UK: Blackwell Science Ltd.

Englyst, K.N., Liu, S. & Englyst, H.N. (2007). Review: Nutritional characterization and measurement of dietary carbohydrates. *European Journal of Clinical Nutrition*, **61**, S19-S39.

Farooq, Z. & Boye, J.I. (2011). Novel food and industrial applications of pulse flours and fractions. In: *Pulse Foods: Processing, Quality and Nutraceutical Applications* (edited by B.K. Tiwari, A. Gowen & B. McKenna). Pp. 283-285. London, UK: Elsevier Inc.

Feldheim, W. & Wisker, E. (2002). Effect of fibre-enriched bread on the glycemic control in diabetes mellitus type 2. *Ernahrung*, **26(5)**, 197-200.

Fernández-Ginés, J.M., Fernández-López, J., Sayas-Barbera, E., Sendra, E. & Pérez-Álvarez, J.A. (2004). Lemon albedo as a new source of dietary fiber: application to bologna sausages. *Meat Science*, **67**, 7-13.

Fernández-López, J., Sendra, E., Sayas-Barberá, M.E., Navarro, C. & Pérez-Álvarez, J.A. (2008). Physicochemical and microbiological profiles of "Salchichón" (Spanicsh dry-fermented sausage) enriched with orange fiber. *Meat Science*, **80**, 410-417.

García, M.L., Dominguez, R., Galvez, M.D., Casas, C. & Selgas, M.D. (2002). Utilization of cereal and fruit fibres in low-fat dry fermented sausages. *Meat Science*, **60(3)**, 227-236.

García, M.L., Cáceres, E. & Selgas, M.D. (2007). Utilization of fruit fibers in conventional and reduced-fat cooked-meat sausages. *Journal of the Science of Food and Agriculture*, **87**, 624-631.

Garcimartin, M., Vidal-Valverde, C., Martinez-Castro, I. & Musgrove, S. (1995). Evaluation of dietary fibre in potato crisps: influence of the analytical method used. *Journal of Food Quality*, **18**, 33-43.

Gómez, M., Ronda, F, Blanco, C.A., Caballero, P.A. & Apesteguía (2003). Effect of dietary fibre on dough rheology and bread quality. *European Food Research & Technology*, **216**, 51-56.

Goñi, I., Díaz-Rubio, M.E., Pérez-Jiménez J. & Saura-Calixto, F. (2009). Towards an updated methodology for measurement of dietary fiber, including associated polyphenols, in food and beverages. *Food Research International*, **42**, 840-846.

Gordon, D.T. & Kubomura, K. (2003). Beverages as delivery systems for nutraceuticals. In: *Beverage Quality and Safety* (edited by T. Voster & P.C. Vasavada). p. 23. USA: CRC Press LLC.

Guillon, F. & Champ, M. (2000). Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International*, **33**, 233-245.

Hillocks, R.J., Bennett, C. & Mponda, O.M. (2012). Bambara nut: A review of utilisation, market potential and crop improvement. *African Crop Science Journal*, **20(1)**, 1-16.

Hipsley, E.H. (1953). Dietary "fibre" and pregnancy toxaemia. *British Medical Journal*, **2**, 420-422 (As cited by AACC, 2001).

Jideani, V.A. & Murevanhema, Y.Y. (2013). A Bambara groundnut milk beverage and probiotic yoghurt produced therefrom and a method for the production thereof. South Africa- Complete Patent 25 January 2013, 2013/00683.

Jideani, V.A. & Diedericks, C.F. (2014). Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*. In: *Antioxidant-Antidiabetic Agents and Human Health* (edited by O. Oguntibeju). Pp. 187-207. Rijeka, Croatia: Intech Publishers.

Johnson, S.K., McQuillan, P.L., Sin, J.H. & Ball, M.J. (2003). Sensory acceptability of white bread with added Australian sweet lupin (*Lupinus angustifolius*) kernel fibre and its glycaemic and insulinaemic responses when eaten as a breakfast. *Journal of the Science of Food and Agriculture*, **83(13)**, 1366-1372.

Jones, J.M. (2014). CODEX-aligned dietary fiber definitions help to bridge the 'fiber-gap'. *Nutrition Journal*, **13(34)**, 1-10.

Koné, M., Paice, A.G. & Touré, Y. (2011). Bambara groundnut [*Vigna subterranea* (L.) Verdc. (Fabaceae)] usage in human health. In: *Nuts and Seeds in Health and Disease Prevention* (edited by V.R. Preedy, R.R. Watson & V.B. Patel). Pp. 192-194. UK: Elsevier, Inc.

Lawal, O.S., Adebowale, K.O. & Adebowale, Y.A. (2007). Functional properties of native and chemically modified protein concentrates from bambara groundnut. *Food Research International*, **40**, 1003-1011.

Linnemann, A.R. (1990). Cultivation of bambara groundnut (*Vigna subterranea* (L.) Verdc.) in Western Province, Zambia: Report of a field study. *Tropical Crops Communication*, **16**, 19 (As cited by Brough *et al.*, 1993).

López-Caballero, M.E., Gómez-Guillén, M.C., Pérez-Mateos, M. & Montero, P. (2005). A functional chitosan-enriched fish sausage treated by high pressure. *Journal of Food Science*, **70(3)**, 166-171.

Mañas, E., Bravo, L. & Saura-Calixto, F. (1994). Sources of error in dietary fibre analysis. *Food Chemistry*, **50**, 331-342.

Mann, J.I. & Cummings, J.H. (2009). Possible implications for health of the different definitions of dietary fibre. *Nutrition, Metabolism and Cardiovascular Diseases*, **19**, 226-229.

Marlett, J.A., McBurney, M.I. & Slavin, J.L. (2002). Position of the American Dietetic Association: health implications of dietary fibre. *Journal of the American Dietetic Association*, **102**, 993-1000.

Meuser, F. (2001). Technological aspects of dietary fibre. In: *Advanced Dietary Fibre Technology* (edited by B.V. McCleary & L. Prosky). Pp. 253, 262-265. UK: Blackwell Science Ltd.

Meyer, D. & Tungland, B. (2001). Non-digestible oligosaccharides and polysaccharides: Their physiological effects and health implications. In: *Advanced Dietary Fibre Technology* (edited by B.V. McCleary & L. Prosky). Pp. 455. UK: Blackwell Science Ltd.

Mkandawire, C.H. (2007). Review of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) production in Sub-Sahara Africa. *Agricultural Journal*, **2(4)**, 464-470.

Mongeau, R. & Brooks, S.P.J. (2003a). Dietary fiber properties and sources. In: *Encyclopedia of Food Sciences and Nutrition*, 2nd Edition (edited by B. Caballero, L. Trugo & P.M. Finglas). Pp. 1813-1823. New York: Elsevier Science Ltd.

Mongeau, R. & Brooks, S.P.J. (2003b). Dietary fiber determination. In: *Encyclopedia of Food Sciences and Nutrition*, 2nd Edition (edited by B. Caballero, L. Trugo & P.M. Finglas). Pp. 1823-1833. New York: Elsevier Science Ltd.

Mpotokwane, S.M., Gaditlhatlhelwe, E., Sebaka, A. & Jideani, V.A. (2008). Physical properties of bambara groundnuts from Botswana. *Journal of Food Engineering*, **89**, 93-98.

Nakamura, A., Takahashi, T., Yoshida, R., Maeda, H. & Corredig, M. (2004). Emulsifying properties of soybean soluble polysaccharide. *Food Hydrocolloids*, **18**, 795-803.

Nakamura, A., Yoshida, R., Maeda, H. & Corredig, M. (2006). Soy soluble polysaccharide stabilization at oil-water interfaces. *Food Hydrocolloids*, **20**, 277-283.

Nakauma, M., Funami, T., Noda, S., Ishihara, S., Al-Assaf, S., Nishinari, K. & Phillips, G.O. (2008). Comparison of sugar beet pectin, soybean soluble polysaccharide, and gum arabic as food emulsifiers. 1. Effect of concentration, pH, and salts on the emulsifying properties. *Food Hydrocolloids*, **22**, 1254-1267.

Ngugi, G.W. (1997). Country Reports: Kenya. Bambara groundnut *Vigna subterranea* (L.) Verdc. Promoting the conservations and use of underutilized and neglected crops 9. In: *Proceedings of the Workshop on Conservation and Improvement of Bambara Groundnut* (*Vigna subterranea* (L.) Verdc.) (edited by J., Heller, F. Begemann & J. Mushonga). November 1995. Harare, Zimbabwe.

Nguy-Ntamag, F.C. (1997). Country Reports: Cameroon. Bambara groundnut *Vigna subterranea* (L.) Verdc. Promoting the conservations and use of underutilized and neglected crops 9. In: *Proceedings of the Workshop on Conservation and Improvement of Bambara Groundnut* (*Vigna subterranea* (L.) Verdc.) (edited by J., Heller, F. Begemann & J. Mushonga). November 1995. Harare, Zimbabwe.

Nti, C.A. (2009). Effects of bambara groundnut (*Vigna subterranea*) variety and processing on the quality and consumer appeal for its products. *International Journal of Food Science and Technology*, **44**, 2234-2242.

Nwanna, L.C., Enujiugha, V.N., Oseni, A.O. & Nwanna, E.E. (2005). Possible effects of fungal fermentation on bambara groundnuts (*Vigna subterranea* (L.) Verdc.) as a feedstuff resource. *Journal of Food Technology*, **3(4)**, 572-575.

Obizoba, I.C. (1983). Nutritive value of cowpea-bambara groundnut rice mixtures in adult rats. *Nutrition Reports International*, **27**, 709-712.

Ozturk, S., Ozboy-Ozbas, O., Javidipour, I. & Koksel, H. (2008). Utilization of sugarbeet fiber and zero-trans interesterified shortenings in cookie production. *Sugar Industry*, **133(11)**, 704-709.

Pale, E., Nacro, M., Vanhaelen, M. & Vanhaelen-Fastré (1997). Anthocyanins from Bambara groundnut (*Vigna subterranea*). *Journal of Agricultural and Food Chemistry*, **45**, 3359-3361.

Pfoertner, H.N. & Fischer, J. (2001). Dietary fibres of lupins and other grain legumes. In: *Advanced Dietary Fibre Technology* (edited by B.V. McCleary & L. Prosky). Pp. 361-366. UK: Blackwell Science Ltd.

Rodríguez, R., Jiménez, A., Fernández-Bolaños, J., Guillén, R. & Heredia, A. (2006). Dietary fibre from vegetable products as source of functional ingredients. *Trends in Food Science & Technology*, **17**, 3-15.

Rodríguez, M.S., Montero, M., Staffolo, M.D., Martino, M., Bevilacqua, A. & Albertengo, L. (2008). Chitosan influence on glucose and calcium availability from yogurt: *in vitro* comparative study with plants fibre. *Carbohydrate Polymers*, **74(4)**, 797-801.

Salem, M.M.E., Abd-El-Gawad, M.A.M., Hassan, F.A.M. & Effat, B.A. (2007). Use of symbiotics for production of functional low-fat labneh. *Polish Journal of Food and Nutrition Science*, **57(2)**, 151-159.

Sánchez-Alonso, I., Haji-Maleki, R. & Borderias, A.J. (2007). Wheat fiber as a functional ingredient in restructured fish products. *Food Chemistry*, **100(3)**, 1037-1043.

Sánchez-Alonso, I., Jimenez-Escrig, A., Saura-Calixto, F. & Borderias A.J. (2008). Antioxidant protection of white grape pomace on restructured fish products during frozen storage. *LWT – Food Science and Technology*, **41(1)**, 42-50.

Sánchez-Zapata, E. & Pérez-Alvarez, J.A. (2008). El aceite de olive un alimento funcional. *Alimentación Equipos y Tecnología*, **233**, 32-35.

Sánchez-Zapata, E., Fernández-López, J., Sendra, E., Sayas-Barbera, M.E., Navarro, C., Viuda-Martos, M. & Pérez-Álvarez, J.A. (2008). Effect of orange fibre on the colour and heme iron content of "paté" elaborated with dark muscle ("Sangacho") of bluefin tuna (*Thunus thynnus*). In: *Proceedings of International Functional Foods Conference*. p. 80. Porto, Portugal: EULAFF/CYTED.

Sanz, T., Salvador, A., Jimenez, A. & Fiszman, S.M. (2008). Yogurt enrichment with functional asparagus fibre. Effect of fibre extraction method on rheological properties, colour, and sensory acceptance. *European Food Research and Technology*, **227(5)**, 1515-1521.

Sasaki,T., Kohyama, K. & Yasui, T. (2004). Effect of water-soluble and insoluble non-starch polysaccharides isolated from wheat flour on the rheological properties of wheat starch gel. *Carbohydrate Polymers*, **57**, 451-458.

Sathe, S.K. & Deshpande, S.S. (2003). Beans. In: *Encyclopedia of Food Sciences and Nutrition*, 2nd Edition (edited by B. Caballero, L. Trugo & P.M. Finglas). Pp. 403-412. New York: Elsevier Science Ltd.

Schaafsma, G. (2004). Health claims, options for dietary fibre. In: *Dietary Fibre* (edited by J.M. van der Kamp, N.G. Asp, J. Miller & G. Schaafsma). Pp. 27-38. The Netherlands: Wageningen Academic Publishers (As cited by Rodríquez *et al.*, 2006).

Schneeman, B.O. (1998). Dietary fibre and gastrointestinal function. *Nutrition Research*, **18**, 625-632.

Sendra, E., Fayos, P., Lario, Y., Fernández-López, J., Sayas-Barbera, E. & Pérez-Álvarez, J.A. (2008). Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiology*, **25(1)**, 13-21.

Staffolo, D.M., Bertola, N., Martino, M. & Bevilacqua, A. (2004). Influence of dietary fiber addition on sensory and rheological properties of yogurt. *International Dairy Journal*, **14(3)**, 263-268.

Stephens, J.M. (2012). Bambara Groundnut — *Voandzeia subterranea* (L.) Thouars. [WWW Document]: URL http://edis.ifas.ufl.edu/mv014. Accessed on 11 June 2013.

Swanevelder, C.J. (1997). Country Reports: South Africa. Bambara groundnut *Vigna subterranea* (L.) Verdc. Promoting the conservations and use of underutilized and neglected crops 9. In: *Proceedings of the Workshop on Conservation and Improvement of Bambara Groundnut* (*Vigna subterranea* (L.) Verdc.) (edited by J., Heller, F. Begemann & J. Mushonga). November 1995. Harare, Zimbabwe.

Swanevelder, C.J. (1998). Bambara—Food for Africa (*Vigna subterranea* – bambara groundnut). Pp. 1-16. South Africa: National Department of Agriculture.

Tan, C. (2004). Beverage emulsions. In: *Food Emulsions 4th Edition, Revised and Expanded* (edited by S.E. Friberg, K. Larsson & J. Sjöblom). Pp. 485-524. New York, USA: Marcel Dekker, Inc.

Tanya, A.K.N., Mbofung, C.M.F. & Keshinro, O.O. (1997). Soluble and insoluble fiber contents of some Cameroonian foodstuffs. *Plant Foods for Human Nutrition*, **51**, 199-207.

Tharanathan, R.N. & Mahadevamma, S. (2003). Grain legumes – a boon to human nutrition. *Trends in Food Science & Technology*, **14**, 507-518.

Tiwari, U. & Cummins, E. (2011). Functional and physicochemical properties of legume fibers. In: *Pulse Foods: Processing, Quality and Nutraceutical Applications* (edited by B.K. Tiwari, A. Gowen & B. McKenna). Pp. 146. London, UK: Elsevier Inc.

Tiwari, B.K., Gowen, A. & McKenna, B. (2011). Introduction. In: *Pulse Foods: Processing, Quality and Nutraceutical Applications* (edited by B.K. Tiwari, A. Gowen & B. McKenna). Pp. 1-2. London, UK: Elsevier Inc.

Tosh, S.M. & Yada, S. (2010). Review – Dietary fibres in pulse seeds and fractions: Characterisation, functional attributes, and applications. *Food Research International*, **43**, 450-460.

Trinidad, T.P., Mallillin, A.C., Valdez, D.H., Loyola, A.S., Askali-Mercado, F.C., Castillo, J.C., Encabo, R.R., Masa, D.B., Maglaya, A.S. & Chua, M.T. (2006). Dietary fiber from coconut flour: a functional food. *Innovative Food Science and Emerging Technologies*, **7(4)**, 302-317.

Trowell, H. (1972). Ischemic heart disease and dietary fiber. *American Journal of Clinical Nutrition*, **25**, 926-932 (As cited by Champ *et al.*, 2003).

Turowski, M., Deshmukh, B., Harfmann, R., Conklin, J. & Lynch, S. (2007). A method for determination of soluble dietary fiber in methylcellulose and hydroxypropyl methylcellulose food gums. *Journal of Food Composition and Analysis*, **20**, 420-429.

Uebersax, M.A. & Occeña, L.G. (2003). Legumes in the diet. In: *Encyclopedia of Food Sciences and Nutrition*, 2nd Edition (edited by B. Caballero, L. Trugo & P.M. Finglas). Pp. 3520-3528. New York: Elsevier Science Ltd.

Uvere, P.O., Uwaegbute, A.C. & Adedji, E.M. (1999). Effects of malting on the milling performance and acceptability of bambara groundnut (*Voandzeia subterranea* Thouars) seeds and products. *Plant Food for Human Nutrition*, **54**, 49-57.

Uysal, H., Bilgiçli, N., Elgun, A., Ibanoglu, S., Herken, E.N. & Demir, M.K. (2007). Effect of dietary fibre and xylanase enzyme addition on the selected properties of wire-cut cookies. *Journal of Food Engineering*, **78(3)**, 1074-1078.

Varga, J., Szigeti, T. & Gyenis, B. (2006). Influence of chicory inulin on the survival of microbiota of a probiotic fermented milk during refrigerated storage. *Annals of Microbiology*, **56(2)**, 139-141.

Vergara-Valencia, N., Granados-Pérez, E., Agama-Acevedo, E., Tovar, J., Ruales, J. & Bello-Pérez, L.A. (2007). Fibre concentrate from mango fruit: characterization, associated antioxidant capacity and application as a bakery product ingredient. *LWT – Food Science and Technology*, **40(4)**, 722-729.

Viuda-Martos, M., Baeze-Rodríguez, M.C., Sayas-Barberá, E., Sendra, E., Fernández-López, J., & Pérez-Álvarez, J.A. (2008). Effecto de la adición de fibra de naranja y aceites esencial de oregano sobre las características físicas, químicas, fisiscoquímicas y sensoriales de un embutido modelo de pasta fina. *Eurocarne*, **166**, 79-90.

Viuda-Martos, M., López-Marcos, M.C., Fernández-López, J., Sendra, E., López-Vargas, J.H. & Pérez-Álvarez, J.A. (2010). Role of fiber in cardiovascular diseases: A review. *Comprehensive Reviews in Food Science and Food Safety*, **9**, 240-258.

Volikakis, P., Biliaderis, C.G., Vamvakas, C. & Zerfiridis, G.K. (2004). Effects of a commercial oat beta-glucan concentrate on the chemical, physico-chemical and sensory attributes of a low-fat, white-brined cheese product. *Food Research International*, **37(1)**, 83-94.

Vural, H., Issa, I. & Ozbas, O.O. (2004). Effects of interesterified vegetable oils and sugar beet fiber on the quality of frankfurters. *Meat Science*, **67(1)**, 65-72.

Wang, J., Rosell, C.M. & Benedito de Barber, C. (2002). Effect of the addition of different fibres on wheat dough performance and bread quality. *Food Chemistry*, **79**, 221-226.

Wang, N. & Toews, R. (2011). Certain physicochemical and functional properties of fibre fractions from pulses. *Food Research International*, **44**, 2515-2523.

Williams, P.A. & Phillips, G.O. (2003). Properties of individual gums. In: *Encyclopedia of Food Sciences and Nutrition*, 2nd Edition (edited by B. Caballero, L. Trugo & P.M. Finglas). Pp. 2992-3001. New York: Elsevier Science Ltd.

Yılmaz, I. & Daglıoglu, O. (2003). The effect of replacing fat with oat bran on fatty acid composition and phytochemical properties of meatmalls. *Meat Science*, **65(2)**, 819-823.

Yusuf, A.A., Ayedun, H. & Sanni, L.O. (2008). Chemical composition and functional properties of raw and roasted Nigerian benniseed (*Sesamum indicum*) and bambara groundnut (*Vigna subterranean*). *Food Chemistry*, **111**, 277-282.

CHAPTER THREE
ISOLATION AND CHARACTERISATION OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* (L.) VERDC.) SOLUBLE AND INSOLUBLE DIETARY FIBRE

Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) [BGN] is a readily available yet underutilised legume seed with high carbohydrate content (63%). To highlight its potential value, insoluble and soluble fibres were isolated from four (black-eye: BLE, red: RED, brown: BRN and brown-eye: BRE) BGN varieties and evaluated for some physicochemical and functional properties. High bulk density of BGN insoluble dietary fibre [BGNIF] (0.82 – 0.86 $g.ml^{-1}$) and BGN soluble dietary fibre [BGNSF] (0.81 - 0.93 $g.ml^{-1}$) could have significant packaging cost saving implications. BLE BGNIF ($L^* = 84.22 \pm 0.02$) and BRE BGNSF ($L^* = 87.63 \pm 0.02$) were significantly [$p \leq 0.05$] high in lightness making them appropriate for use in bakery products where a lighter colour is preferred. Yellowness (b^*) ranged from 8.62 – 16.16 for BGNIF and from 6.30 – 7.33 for BGNSF. Combined with the hue angles ranging from 69.80 – 78.17° for BGNIF and 68.11 – 78.92° for BGNSF, colour parameters indicated a yellowish-red colour for BGNIF varieties and a light yellow colour for BGNSF varieties. Polyphenols were significantly [$p \leq 0.05$] highest in RED (13.81 ± 4.20 $mg.g^{-1}$ gallic acid equivalents [GAE]) and BRN (15.56 ± 2.24 $mg.g^{-1}$ GAE) BGNIF and corresponding condensed tannins of 0.100 ± 0.005 $mg.g^{-1}$ and 0.160 ± 0.007 $mg.g^{-1}$, respectively. Polyphenol content were highest in BGNSF ranging from 45.42 – 55.90 $mg.g^{-1}$ GAE, indicative of their potential antioxidant activity. Arabinose/galactose (31.04 – 37.12%), xylose (16.53 – 27.30%) and mannose (14.48 – 22.24%) were identified as the major sugars in BGNIF, whereas the major sugars in BGNSF were xylose (38.52 – 54.47%) and mannose (24.90 – 37.45%). Swelling capacity was significantly [$p \leq 0.05$] highest for BRE BGNIF (7.72 ± 0.49 $ml.g^{-1}$), a value higher than commercial fibres playing an important role in satiety levels. Water retention capacity was 1.63 – 2.01 g $water.g^{-1}$ dry weight with no significant [$p > 0.05$] differences observed between the four varieties. Fat absorption for BRN BGNIF (1.52 ± 0.04 g $oil.g^{-1}$ dry weight) differed significantly [$p \leq 0.05$] from the RED BGNIF (1.38 ± 0.01 g $oil.g^{-1}$ dry weight) being the lowest, whilst fat absorption for BGNSF ranged from 4.04 – 4.55 g $oil.g^{-1}$ dry weight. Compared to commercial pea fibres, all BGN fibres had higher fat absorption capacity, thus indicating the importance of BGNIF and BGNSF in food applications. BRN BGNIF was significantly [$p \leq 0.05$] higher in viscosity at 5% and 7% levels. All BGNIF varieties were characterised by low viscosities, making them ideal to be used in non-viscous food systems. BGN insoluble and soluble fibres showed positive physicochemical and functional properties, in many cases comparable or more beneficial than commercial fibres.

3.1 Introduction

Dietary fibre, the term which first appeared in 1953 and which gained wide interest in the mid 1970's due to its role in health and nutrition (De Vries, 2003), can be classified amongst the important ingredients to be included in the diet (Schaafsma, 2004). The increased intake of dietary fibre has led to the reduced risk of the development of several diseases such as diabetes, coronary heart disease, obesity and some forms of cancer (Mann & Cummings, 2009). As reviewed by Anderson *et al.* (2009), the consumption of high levels of dietary fibre leads to several beneficial physiological effects. Apart from the physiological functionalities, dietary fibre also imparts several technological properties when incorporated into foods. These include amongst others water holding capacity, oil holding capacity, water solubility, viscosity, gel-forming capacity and antioxidant capacity (Elleuch *et al.*, 2011).

Dietary fibre can be classified by numerous ways including on the basis of their structure, properties and their applications. The conventional classification of dietary fibre however, is done on the basis of their water solubility and includes two categories; soluble dietary fibre [SDF] and insoluble dietary fibre [IDF] (Chawla & Patil, 2010). SDF forms a solution when mixed with water and includes mucilage, gums and pectic substances. IDF on the other hand do not form a solution when mixed with water and includes cellulose, some hemicelluloses and lignin (Elleuch *et al.*, 2011; Chawla & Patil, 2010). Both fractions show differences in their physiological and technological properties (Jiménez-Escrig & Sánchez-Muniz, 2000; Roehrig, 1988). Therefore, when characterising a dietary fibre, as with any other polysaccharide, several functional/ physicochemical properties are investigated as is described elsewhere (Elleuch *et al.*, 2011; Tosh & Yada, 2010; Eltayeb *et al.*, 2011; Adebowale *et al.*, 2002).

Dietary fibre measurement in foods is seen as a complex issue, related to the lack of a unique definition of fibre; consequently numerous methods for the determination of dietary fibre has been developed (Elleuch *et al.*, 2011; Rodríguez *et al.*, 2006). These methods can be classified into two broad categories namely enzymatic-gravimetric methods and enzymatic-chemical methods (Elleuch *et al.*, 2011; Rodríguez *et al.*, 2006; Mongeau & Brooks, 2003a). The enzymatic-gravimetric methods measure indigestible polysaccharides, some resistant starch, lignin and associated compounds such as phenolic compounds and Maillard reaction products (Englyst *et al.*, 2007; Elleuch *et al.*, 2011). The key analytical principles involved include partial enzymatic hydrolysis of protein and starch, followed by precipitation of the residue in 80% ethanol and isolation by filtration, total residue weight is recorded and determined by correcting the protein and ash contents (Englyst *et al.*, 2007). The enzymatic-chemical methods measure the non-starch polysaccharides and Klason lignin to obtain a quantity for total dietary fibre (Elleuch *et al.*, 2011). The analytical principles involved are the complete dispersion of starch and its enzymatic hydrolysis. The residue is

precipitated with 80% ethanol and isolated by centrifuging. The non-starch polysaccharides are hydrolysed and measured as constituent sugars by chromatography or colorimetry (Englyst *et al.*, 2007). The most commonly used methods to measure dietary fibre in foods is the AOAC gravimetric-enzymatic method and the Englyst enzymatic-chemical method. In comparison the AOAC method measures the highest fibre content and the Englyst method the lowest (Goñi *et al.*, 2009; Elleuch *et al.*, 2011). As investigated by Mañas *et al.* (1994) however, these dietary fibre analytical methods may both be affected by errors and as such are not accurate enough for either scientific or commercial purposes. Improvements to these methods have been recommended which includes centrifugation after enzymatic treatments to separate the IDF residues and supernatants, followed by dialysis of the supernatants to obtain the SDF fractions. Both fractions are subjected to acid hydrolyses before chemical quantification. Compared to ethanolic precipitation, dialysis was found to be more reliable, as incomplete precipitation of SDF fractions and co-precipitation of non-fibre components were obtained with the former method. Some polysaccharides are also not detected by existing methods due to temperature and solvent incompatibility (Mañas *et al.*, 1994; Turowski *et al.*, 2007). Goñi *et al.* (2009) found substantial amounts of dietary polyphenols and resistant protein associated with both IDF and SDF, thus supporting the inclusion of these compounds in dietary fibre measurement. The methodology used in this study was therefore based on these recommended improvements.

The recommended daily dietary fibre intake ranges from 30 – 40 g/ day, with suggestions made by most nutritionists and dieticians that 20 – 30% of dietary fibre intake should be from soluble dietary fibre (Chawla & Patil, 2010; Elleuch *et al.*, 2011). Amongst the most important food sources of dietary fibre are the pulses which are the edible seeds of leguminous crops (Tosh & Yada, 2010). By expanding the utilisation of pulse derived fibres, human health can be enhanced as well as an increase in the market value of the crops (Wang & Toews, 2011; Tosh & Yada, 2010). Wang & Toews (2011) reported that pulse fibres have superior functional properties and this leads to a great potential for their use in various food applications. Commercially, cotyledon and hull fibres from peas are available for food applications; however fibres from other pulse sources are scarce.

A potential pulse fibre source is the Bambara groundnut [BGN] (*Vigna subterranea* (L.) Verdc.) – an under-utilised legume seed cultivated throughout Africa, Indonesia, Malaysia and Sri Lanka (Eltayeb *et al.*, 2011). BGN have a rich nutritional profile which indicates to the potential of sustaining the dietary needs of many people in both urban and rural communities (Jideani & Diedericks, 2014). Considered as a good source of total dietary fibre [TDF] (26.5 g/100 g dry weight), the isolation and investigation of BGN dietary fibre fractions remain to be explored. There is also a need in the food industry to obtain new dietary fibre food sources due to a rapid growth in the market for products and ingredients rich in dietary fibre. The fractionation of BGN into dietary fibre fractions could therefore

address the need and fill the gap in the market for better utilisation of this under-valued crop, and as a result increase its market value and present a new source of fibre to the food industry. The objectives of this study were therefore to isolate and characterise the insoluble and soluble dietary fibre fractions from BGN with a view to establish their potential for use in food applications.

3.2 Materials and Methods

The overview of the methodology employed in this chapter is shown in Figure 3.1. BGN flour were obtained from four varieties (black-eye, red, brown and brown-eye) and analysed for total dietary fibre [TDF] content. Insoluble and soluble dietary fibre were extracted from the four flour samples and evaluated for several physicochemical and functional properties which included polyphenolic content, uronic acids and neutral sugars composition, colour, bulk density, microstructure, fat absorption capacity, hydration properties and apparent viscosity. Detailed descriptions of the methodology are given in subsequent sections.

3.2.1 Source of materials

BGN seeds were purchased from Thusano Products (Louis Trichardt, South Africa). Enzymes including amyloglucosidase (A9913), α-amylase (A3176) and pancreatin (P7545) were obtained from Sigma Aldrich Co. (St. Louis, Missouri, USA); pepsin (1.07190) was obtained from Merck KGaA (Darmstadt, Germany). All reagents used in this study were of analytical grade.

3.2.2 Production of BGN flour and total dietary fibre determination

The BGN seeds were screened to eliminate defective seeds and foreign materials such as dirt, dust and immature seeds; followed by sorting into four varieties on the basis of seed colour – black-eye, brown, red and brown-eye. The sorted seeds were washed, dried and milled to powder (0.50 mm mesh) by using a hammer mill (TRF 400, Trapp, Brazil). The powdered seeds (flour) were stored in airtight polyethylene bags at a temperature of 4°C prior to analyses. All BGN flour varieties were analysed for TDF content by the Official Method 991.43 (AOAC, 2005).

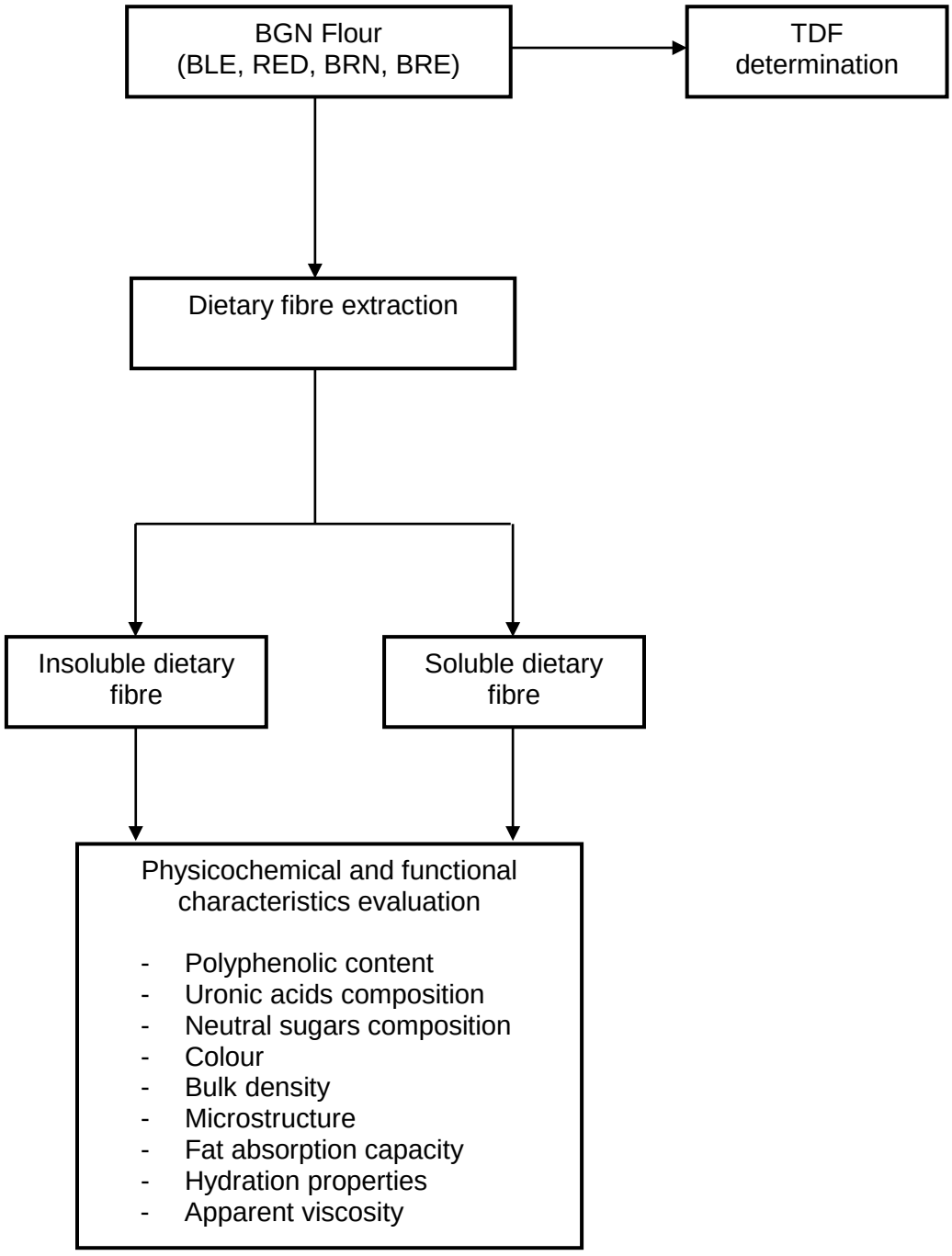


Figure 3.1 Methodology overview. BGN: Bambara groundnut, BLE: black-eye, RED: red, BRN: brown, BRE: brown-eye, TDF: total dietary fibre.

3.2.3 Dietary fibre extraction

The updated methodology for extraction of dietary fibre as described by Goñi *et al.* (2009) was adopted in this study with modification. Figure 3.2 illustrates the analytical scheme for insoluble and soluble dietary fibre extraction. BGN flour (18 g) was weighed into 2 L Schott bottles and 600 ml phosphate buffer (pH 7.5) was added to each bottle. The pH was adjusted to pH 7.5 (Figure 3.2a). Pepsin solution (12 ml) was added to each bottle and the solutions incubated in a water bath at 40°C for 1 hr. The pH was adjusted to pH 7.5 by the addition of ± 30 ml NaOH solution. Pancreatin solution (60 ml) was added to each bottle and the solutions incubated at 37°C for 6 h. After incubation 600 ml trizma-maleate buffer (pH 6.9) was added to each bottle, the pH of the solutions measured and adjusted to pH 6.9 followed by the addition of 60 ml α-amylase solution and incubation at 37°C for 16 h with continuous agitation (Figure 3.2b). After incubation the solutions were transferred to 250 ml centrifuge tubes and samples centrifuged at 3000 x g for 15 min and the supernatants removed. The residues were washed twice with 5 ml distilled water and all supernatants combined (Figure 3.2c). The residues were vacuum-dried for 2 h at 105°C, cooled in a desiccator and the weight recorded to determine the residue weight; which indicates the IDF fraction. The dried IDF were ground to a fine uniform powder (0.355 mm mesh) and stored at 4°C prior to analyses (Figure 3.2d). Sodium acetate buffer (pH 4.75) with a volume of 200 ml was added to the supernatants followed by the addition of 2 ml amyloglucosidase, and incubation in a water bath at 60°C for 45 min with constant shaking (Figure 3.2e). After incubation, the supernatant solutions were subjected to a tangential flow filtration system (diafiltration) (Figure 3.2f). Each solution was washed with four diafiltration volumes water and the contaminants removed through the hollow fiber filtration module with molecular weight cut-off 10 kD (Spectrum Laboratories Inc., USA). The sample retentate was freeze-dried overnight and quantified gravimetrically as the SDF fraction (Figure 3.2g). The dried SDF samples were stored at 4°C prior to analyses.

3.2.4 Determination of some physicochemical and functional properties of BGN insoluble and soluble dietary fibre

Microstructure of fibre fractions

The microstructure of the samples was examined by scanning electron microscopy (SEM) and measurement proceeded as described in Rosell *et al.* (2009). Powdered BGNIF samples of each variant were carefully placed on carbon adhesives attached to aluminium stubs. All samples were sputter-coated with gold and palladium to ensure conductivity of the samples, followed by analysis with a field emission gun SEM (Model FEG HR SEM, Carl Zeiss, Germany) performed at an accelerating voltage of 10 kV.

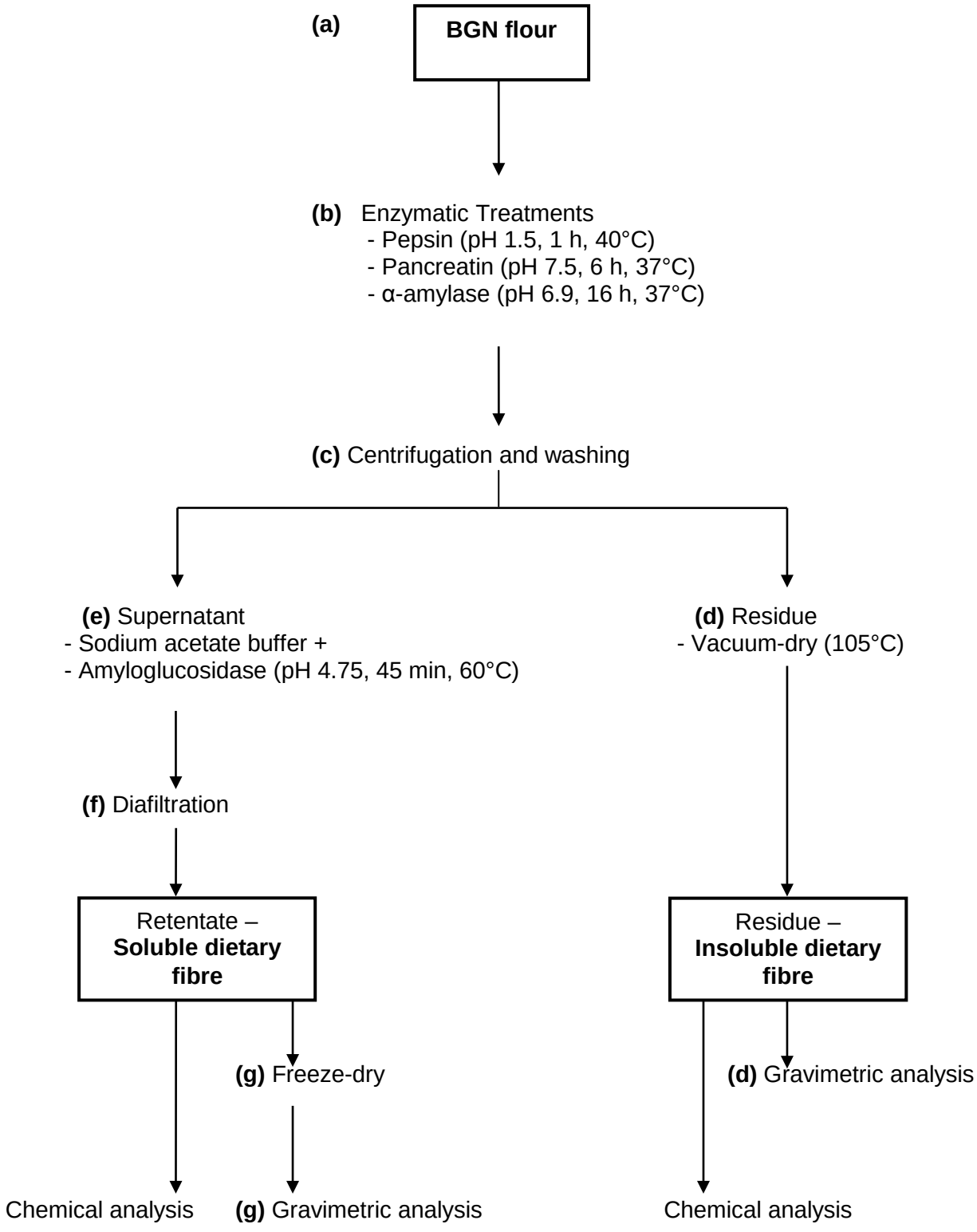


Figure 3.2 Schematic representation of dietary fibre isolation [Adapted from Goñi *et al.*, 2009].

Bulk density

The method as described by Parrott & Thrall (1978) was used to determine bulk density of the fibres. Briefly, 2 g of each fibre was placed into a graduated syringe and sufficient pressure applied to pack the contents in the syringe. The final volume of the fibre in the syringe was recorded and the bulk density expressed as grams per millilitre ($g.ml^{-1}$).

Colour of BGN fibre fractions

The colour of the BGN fibre fractions was measured with a spectrophotometer (Model CM-5, Konica Minolta Sensing, Japan) using the CIE-L*a*b* and L*C*h colour space systems. The instrument was calibrated by using the white calibration plate followed by zero calibration. Powdered samples were placed evenly in the provided petri-dish (diameter 30 mm) covering the bottom of the dish, to allow for reflectance measurement. Measurements for each sample were performed in triplicate at three different positions in the samples (one reading = average of three readings per rotated position), with the results recorded in L* (lightness), a* (chromaticity coordinate +a* = red and –a* = green), b* (chromaticity coordinate +b* = yellow and –b* = blue), C* (chroma) and h (hue angle 0° = +a*, 90° = +b*, 180° = -a* and 270° = -b*) [SpectraMagic NX, version CM-S100w 2.03.0006, Konica Minolta, 2010].

Polyphenolic compounds in BGN fibres

Condensed tannins (proanthocyanidins) and the hydrolysable polyphenolics (PPs) were determined in the BGNIF residues, and associated PPs were determined in the BGNSF permeates. For determination of the condensed tannins, the BGNIF residues were treated with 5 ml/l HCl-Butanol at 100°C for 1 hr and the condensed tannins calculated from the anthocyanidin solutions absorbance at 555 nm. A standard curve of concentration (ppm) = 0.0072 and absorbance +0.0072 was used for quantification of condensed tannins (Goñi *et al.*, 2009).

For determination of the hydrolysable PPs, 20 ml of methanol and 2 ml of sulphuric acid were added to the BGNIF residues followed by incubation at 85°C for 20 h. The Folin-Ciocalteu assay was used for analysis of the PPs, by mixing 0.5 ml of hydrolysate with 0.5 ml of Folin-Ciocalteu reagent and adding 10 ml of Na_2CO_3 solution (75 $g.L^{-1}$) after 3 min with thorough mixing. Additional distilled water was added and thoroughly mixed by inverting the tubes a few times. The absorbance was measured at 750 nm on a spectrophotometer using a standard curve prepared with gallic acid, and results expressed as milligrams per gram gallic acid equivalents ($mg.g^{-1}$ GAE) (Hung & Yen, 2002).

The BGNSF permeates obtained after ultrafiltration was used for determination of PPs, by using 0.5 ml of BGNSF permeate and following the Folin-Ciocalteu method as described for the determination of BGNIF hydrolysable PPs.

Determination of uronic acids [UA] and neutral sugars [NS] in BGN fibres

Both the BGNIF residues and BGNSF permeates were subjected to hydrolyses before UA and NS determination. The BGNIF residues were hydrolysed with 12 M H_2SO_4 (30°C, 1 hr) and then diluted to 1 M H_2SO_4 (100°C, 1.5 h) with shaking to yield monomers constituents. Samples were centrifuged (3000 x g, 15 min) after acid hydrolysis, the residues washed twice with distilled water and the supernatants combined for UA and NS determination. The BGNSF permeates were hydrolysed with 1 M H_2SO_4 (100°C, 1.5 h) and the hydrolysate subjected to UA and NS determination. *UA* and *NS* were measured in the hydrolysates by spectrophotometry at 340 nm, using the K-Uronic, K-Fucose, K-Arga, K-Mangl, K-Rham and K-Xylose assay kits as supplied by Megazyme International (Ireland).

Hydration properties

Swelling capacity [SC]: The method as described by Wang & Toews (2011) was adopted for measurement of the SC of the fibre fractions. A sample weight of 200 mg was weighed in triplicate into 10 ml graduated volumetric cylinders (10.5 mm diameter). The samples were hydrated for 18 h with 10 ml of distilled water, added at 2 ml increments whilst gently stirring to disperse the sample without lump formation. The bed volume was recorded after equilibrium, and the SC expressed as the volume occupied by the hydrated sample per gram of the original dry sample ($ml.g^{-1}$).

Water retention capacity [WRC]: As described by Wang & Toews (2011), WRC of the fibre fractions was measured by hydrating 1 g of sample with 30 ml distilled water in a 50 ml centrifuge tube. After equilibrium (18 h), the samples were centrifuged (3000 x g, 20 min) and the supernatant decanted. The pellet was then allowed to drain by carefully inverting the tubes for 10 min and the final weight recorded. WRC was expressed as the amount of water retained per gram dry sample (g $water.g^{-1}$ dry weight).

Fat absorption [FA]

FA was measured according to the method of Wang & Toews (2011). Fibre (1 g) was added to 5 ml canola oil in a 50 ml centrifuge tube. The content was vortexed for 15 sec every 5 min, and after 20 min the tubes were centrifuged (1600 x g, 25 minutes) at room temperature. Excess oil was decanted and the tubes weighed. FA is expressed as the amount of absorbed oil per gram of sample (g $oil.g^{-1}$ dry weight).

Apparent viscosity

The method as described by Abdul-Hamid & Luan (2000) was adopted for viscosity measurement of BGNIF varieties. The samples were prepared into slurries of four different concentrations (1%, 3%, 5% and 7%) by slowly adding the appropriate sample weight to the distilled water, followed by mixing in a Waring blender at high speed for 1 min. Each solution

was left at room temperature for 24 h, to allow for equilibrium and entrapped air to escape. Viscosity measurement was done at room temperature using a rheometer (Rheolab MC 1, Paar Physica GmbH, Austria) with spindle 23 DIN (diameter = 25 mm) at shear rates of 500 – 1000.s^{-1}.

3.2.5 Statistical analysis

Statistical analysis was performed by testing significant differences between treatments using multivariate analysis of variance and Duncan's multiple range test was used to separate means where differences existed. Principal Component Analysis [PCA] was applied to extract the components that explained the variability in the BGNIF and BGNSF physicochemical and functional properties (IBM SPSS Statistics, version 22, 2013).

3.3 Results and Discussion

3.3.1 TDF content of BGN flour and yield of BGN fibres

The four (black-eye, red, brown, brown-eye) BGN flour varieties were characterised by high TDF content. Red (24.3 ± 1.4% dry matter) and black-eye (23.9 ± 0.3% dry matter) BGN were significantly [$p \leq 0.05$] higher in TDF compared to brown-eye BGN (17.7 ± 0.7%) with lowest TDF. TDF for brown BGN was 21.0 ± 1.1% dry matter. These values confirm the potential of the different BGN varieties as good fibre sources.

Insoluble and soluble dietary fibres were successfully isolated from the four BGN varieties (Figure 3.3). Brown-eye (37.4 ± 1.6%) and black-eye (39.4 ± 1.6%) BGNIF had the lowest significant [$p \leq 0.05$] yield, whilst red (48.3 ± 1.0%) BGNIF had the highest significant [$p \leq 0.05$] yield. BGNSF yield ranged from 12.7 – 13.9%. There was no significant [$p > 0.05$] difference in yield between the soluble fibres from different varieties. The yield from BGNSF fractions were clearly lower compared to the insoluble fibre fractions, with an average ratio of 1:3 for BGNSF to BGNIF. The higher BGNIF yield was expected as the insoluble fibre content of most legumes is comparably higher than the soluble fibre content (Tiwari & Cummins, 2011). Dalgetty & Baik (2003) also found that insoluble fibre constitutes a major part of legume hulls; this further explains the higher BGNIF yield since the whole seeds (hulls and cotyledons) were milled to obtain flour from which the fibre fractions were extracted. Furthermore, the objective of isolating dietary fibres from BGN was achieved.

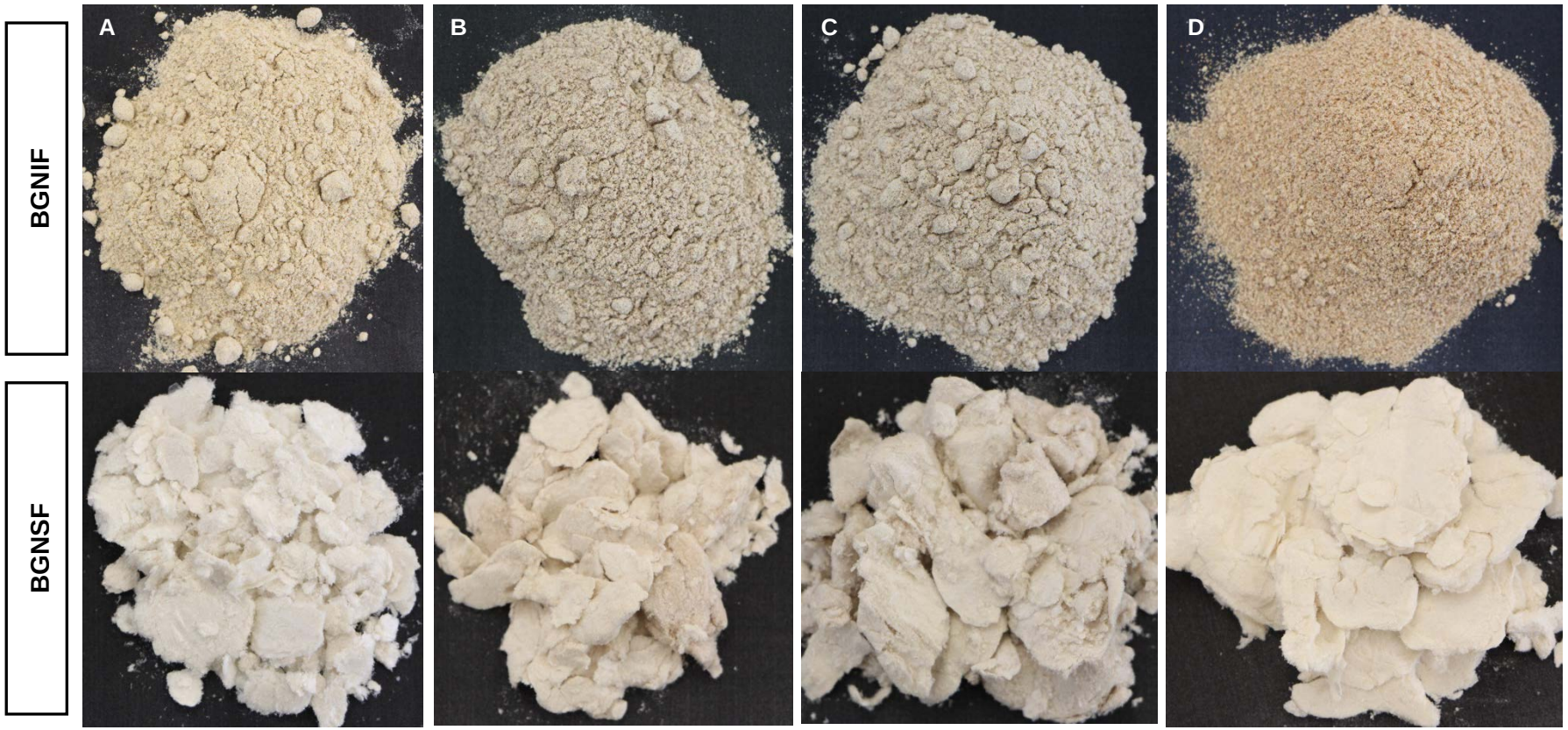


Figure 3.3 Insoluble [BGNIF] and soluble [BGNSF] dietary fibres isolated from four Bambara groundnut [BGN] varieties. **A:** black-eye, **B:** red, **C:** brown, **D:** brown-eye.

3.3.2 Physicochemical properties of BGN fibres

The physicochemical properties of dietary fibres are of great importance for determining the functionality of a specific fibre in a certain food product (Rosell *et al.*, 2009; Tosh & Yada, 2010). In this study the physical properties evaluated included bulk density and colour parameters, and the chemical properties were the polyphenolic composition and monomer constituents of the BGN fibres.

Microstructure of BGN insoluble fibres

Scanning electron micrographs [SEM] of BGNIFs are shown in Figure 3.4. SEM allows for the visual observation of the morphology and structure of the fibre particle size; and as noted by Daou & Zhang (2011) SEM analysis proves useful in studying the physicochemical properties of dietary fibres. All four varieties of BGNIF had particles with irregular shapes and of different sizes. Brown BGNIF consisted of larger particles and in comparison black-eye BGNIF consisted of smaller particles. Compared to commercial fibres, BGNIF particles visually correspond to that of apple fibre as seen in Rosell *et al*. (2009).

Bulk density of BGN fibre fractions

The bulk density of BGN fibres are shown in Table 3.1. Bulk density can be described as the weight of fibre per unit volume (expressed as $g.ml^{-1}$) (Tiwari & Cummins, 2011). Bulk density for BGNIFs ranged from 0.82 – 0.86 $g.ml^{-1}$. No significant [$p > 0.05$] difference was observed between the bulk densities of the black-eye, red and brown BGNIF and the red, brown and brown-eye BGNIF. However, bulk density of black-eye (0.82 ± 0.02 $g.ml^{-1}$) BGNIF differed significantly [$p \leq 0.05$] from the brown-eye (0.86 ± 0.02 $g.ml^{-1}$) BGNIF with the highest bulk density. Bulk density of brown (0.93 ± 0.09 $g.ml^{-1}$) BGNIF was significantly [$p \leq 0.05$] higher compared to black-eye (0.82 ± 0.02 $g.ml^{-1}$) and brown-eye (0.81 ± 0.05 $g.ml^{-1}$) variants. Compared to results reported by Dalgetty & Baik (2003), the bulk density of BGNIF varieties is higher compared to that of pea, lentil and chickpea insoluble fibres [IDF] with the highest bulk density measured for lentil IDF (0.36 $g.ml^{-1}$). It was noted however that in the study by Dalgetty & Baik (2003) the fibre fractions analysed were obtained from the cotyledons of the legume seeds as opposed to being isolated from the whole seed (both hull and cotyledon); therefore making it difficult to compare with the results in this study.

Bulk density of BGNSFs were similar to their insoluble counterparts, ranging from 0.81 – 0.93 $g.ml^{-1}$. Bulk density for brown (0.93 ± 0.09 $g.ml^{-1}$) BGNSF differed significantly [$p \leq 0.05$] from brown-eye (0.81 ± 0.05 $g.ml^{-1}$) and black-eye (0.82 ± 0.02 $g.ml^{-1}$) BGNSF. Dalgetty & Baik (2003) reported bulk density of 0.80 – 0.83 $g.ml^{-1}$ for soluble cotyledon legume fibres, which are comparable to the bulk density of black-eye and brown-eye BGNSF.

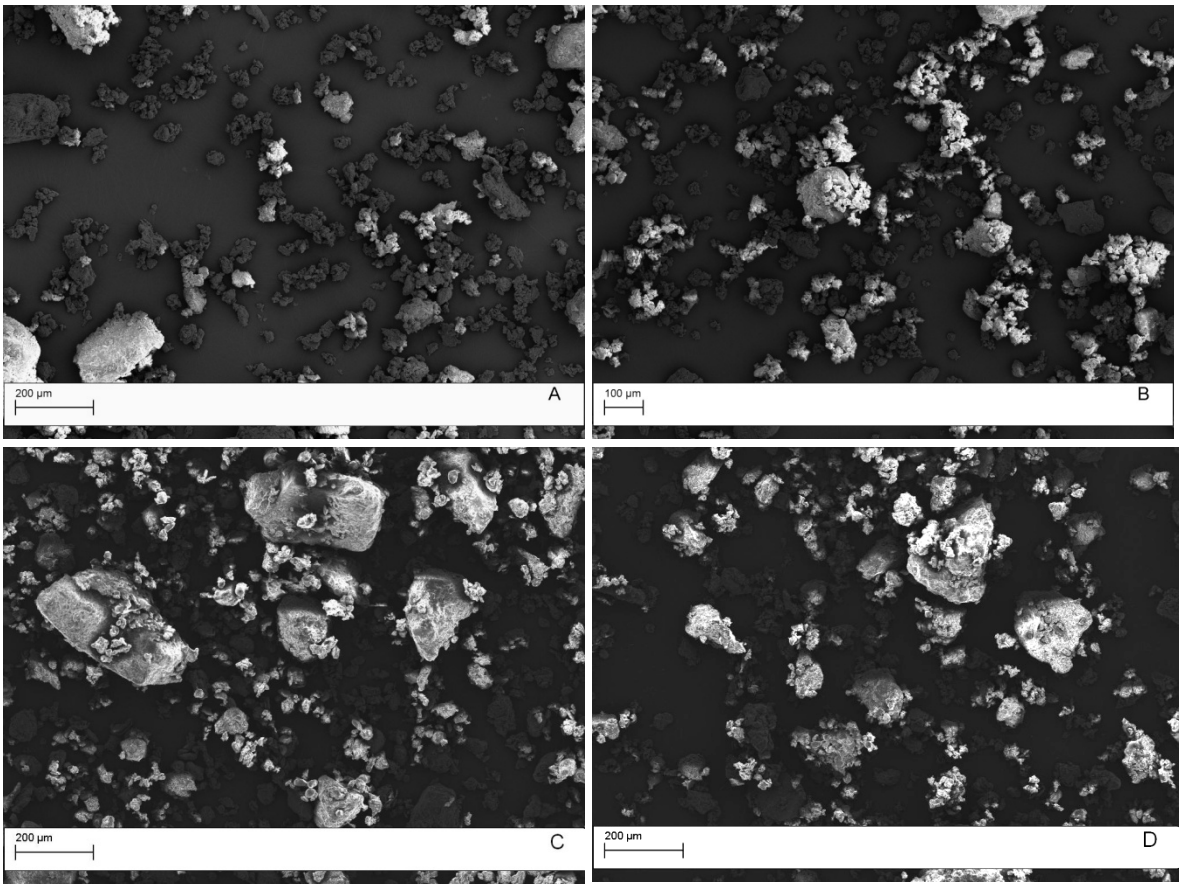


Figure 3.4 Scanning electron micrographs of Bambara groundnut insoluble dietary fibres – **A:** black-eye, **B:** red, **C:** brown, **D:** brown-eye (magnification x200).

Table 3.1 Physical characteristics of Bambara groundnut insoluble and soluble dietary fibres[1, 2]

		Colour parameters				
	BD (g.ml^{-1})	**L***	**a***	**b***	**C***	**h (angle)**
BGNIF varieties						
BLE	0.82 ± 0.02^{a}	84.22 ± 0.02^{a}	2.38 ± 0.01^{a}	8.62± 0.02^{a}	8.95 ± 0.02^{a}	74.56 ± 0.03^{a}
RED	0.83 ± 0.00^{ab}	68.88 ± 0.03^{b}	5.63 ± 0.01^{b}	16.16 ± 0.07^{b}	17.11 ± 0.07^{b}	70.79 ± 0.10^{b}
BRN	0.83 ± 0.00^{ab}	68.77 ± 0.01^{c}	5.40 ± 0.01^{c}	14.67 ± 0.02^{c}	15.63 ± 0.02^{c}	69.80 ± 0.03^{c}
BRE	0.86 ± 0.02^{b}	73.56 ± 0.01^{d}	3.30 ± 0.01^{d}	15.75 ± 0.02^{d}	16.09 ± 0.02^{d}	78.17 ± 0.05^{d}
BGNSF varieties						
BLE	0.82 ± 0.02^{a}	86.64 ± 0.04^{a}	1.23 ± 0.01^{a}	6.30 ± 0.02^{a}	6.42 ± 0.02^{a}	78.92 ± 0.05^{a}
RED	0.88 ± 0.02^{ab}	84.53 ± 0.04^{b}	2.81 ± 0.01^{b}	6.98 ± 0.01^{b}	7.52 ± 0.01^{b}	68.11 ± 0.02^{b}
BRN	0.93 ± 0.09^{b}	83.13 ± 0.02^{c}	2.45 ± 0.00^{c}	6.33 ± 0.00^{c}	6.79 ± 0.00^{c}	68.82 ± 0.01^{c}
BRE	0.81 ± 0.05^{a}	87.63 ± 0.02^{d}	1.64 ± 0.01^{d}	7.33 ± 0.00^{d}	7.51 ± 0.00^{b}	77.41 ± 0.08^{d}

[1] Values are Mean ± Standard deviation. Means within a column followed by the same letter (under the same heading) are not significantly [p > 0.05] different.
[2] BD: bulk density; L*: lightness; a*: red (+a*) to green (-a*) range; b*: yellow (+b*) to blue (-b*) range; C*: chroma; h: hue angle. BGNIF: Bambara groundnut insoluble dietary fibre; BGNSF: Bambara groundnut soluble dietary fibre; BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye.

The bulk density of a fibre is influenced by properties such as the size and distribution of the fibre particles, structural characteristics and possibly the protein concentration in the fibre (Benítez *et al.*, 2013; Dalgetty & Baik, 2003). Bulk density is an important property to consider for both physical (with regards to packaging considerations) and certain physiological reasons. Physiologically, the bulking effect of a fibre can assist in the reduction of a food's energy density which consequently affects satiety levels (Buttriss & Stokes, 2008). Fibres with a lower bulk density would positively contribute to the bulking effect of a fibre in the intestinal tract; due to a larger exposed surface area and polar groups which would lead to a higher swelling volume of the fibre (Lan *et al.*, 2012). An increased surface area will also influence the availability of a fibre in the colon for microbial degradation (Tosh & Yada, 2010). Compared to fibres with a lower bulk density, BGN soluble and insoluble fibres with their higher bulk densities would occupy less packaging space which could have significant cost saving implications.

Colour characteristics of BGN fibre fractions

The colour parameters of BGN fibre fractions are shown in Table 3.1. The BGNIF varieties differed significantly [$p \leq 0.05$] across all colour parameters ranging from 68.77 – 84.22 for lightness, 2.38 – 5.63 for redness, 8.62 – 16.16 for yellowness, 8.95 – 17.11 for chroma and 69.80 – 78.17° for hue angle. Black-eye ($L^* = 84.22 \pm 0.02$) BGNIF was significantly [$p \leq 0.05$] lighter in colour compared to the other varieties. L^* values of food ingredients are important in especially bakery products as a result of consumer preferences (Rosell *et al.*, 2009), where a lighter colour is generally preferred. Red ($a^* = 5.63 \pm 0.01$) BGNIF had significantly [$p \leq 0.05$] highest redness (a^*) which highlights the contributing role of the colour pigments of the seeds on the colour of the fibres. The yellowness (b^*) differed significantly [$p \leq 0.05$] between the different BGNIFs, with brown ($b^* = 14.67 \pm 0.02$) BGNIF exhibiting yellowness similar to that of a commercial pea fibre ($b^* = 14.00$) (Wang & Toews, 2009). Chroma (C^*) ranged from the lowest 8.95 ± 0.02 for black-eye BGNIF to the highest 17.11 ± 0.07 for red BGNIF, thus indicating a more vivid colour for the red BGNIF in comparison to the other three varieties. The hue angle for brown BGNIF ($h = 69.80 \pm 0.03°$) was the lowest and differed significantly [$p \leq 0.05$] from the brown-eye variant ($h = 78.17 \pm 0.05°$) with the highest hue angle. Hue angles for all BGNIFs were closest to the yellow chromaticity coordinate ($+b^* = 90°$). Compared to commercial pea fibres with redness ranging from -0.98 – 0.53 and yellowness ranging from 6.9 – 14.0 (Wang & Toews, 2011), and with high hue angles between 69.80 – 78.17°, BGNIF varieties are more yellowish-red in colour.

The BGNSF colour parameters ranged from 83.13 – 87.63 for lightness, 1.23 – 2.81 for redness, 6.30 – 7.33 for yellowness, 6.42 – 7.52 for chroma and 68.11 – 78.92° for hue angle with significant [$p \leq 0.05$] differences between all varieties across all parameters. Brown-eye ($L^* = 87.63 \pm 0.02$) BGNSF differed significantly [$p \leq 0.05$] from the other varieties

with the highest lightness. Wang & Toews (2011) reported lightness of 86.2 for a commercial pea fibre; lightness of black-eye (L* = 86.64 ± 0.04) BGNSF is thus comparable to this pea fibre. The redness (a*) for all BGNSF varieties are similar to the redness observed for lentil fibre varieties (a* = 1.28 – 2.92) as reported by Wang & Toews (2011). The yellowness (b*) differed significantly [$p \leq 0.05$] between all BGNSF's, with red (b* = 6.98 ± 0.01) BGNSF exhibiting yellowness similar to that of a commercial pea hull fibre (b* = 6.90) (Wang & Toews, 2009). When comparing colour parameters of the BGN fibre fractions, it was noted that the BGNSF fractions have lower redness, yellowness and chroma values, higher lightness and similar hues compared to BGNIF fractions. Chroma of red (7.52 ± 0.01) and brown-eye (7.51) BGNSF was the highest and differed significantly from the brown-eye and brown varieties. All BGNSF varieties were also characterised with hue values closest to the yellow chromaticity coordinate, with the highest hue angle observed for black-eye (h = 78.92 ± 0.05) BGNSF. The higher lightness and hue angles indicate that BGNSF varieties were light yellow in colour.

Wang & Toews (2011) noted that colour differences observed between different fibre fractions could be attributed to the different coloured pigments found in legumes. This explains the difference across all colour parameters for the BGN fibres, as all seeds are characterised by different colours. The inclusion of dietary fibres in many food applications are to an extent determined by the colour of fibre as this impacts sensory characteristics (Tosh & Yada, 2010). Depending on the fibre colour, the quantities that can be added to food products are limited due to undesirable colour changes which may occur (Elleuch *et al.*, 2011). Dietary fibres are most commonly added to bakery products to extend freshness, improve bread crumb and texture characteristics and for modification of loaf volumes (Elleuch *et al.*, 2011). With a significantly higher lightness value (L* = 84.22 ± 0.02) and a hue angle of 74.56 ± 0.03, black-eye BGNIF could possibly be incorporated into bakery products without causing adverse colour effects. BGNSF varieties could also find applications in bakery products for the same reasons. Red BGNIF with the higher red (a* = 5.63 ± 0.01) and more vivid colour (C* = 17.11 ± 0.07), could possibly find applications in meat (e.g. beef burgers, sausages) and fruit products (e.g. strawberry jam) where a red/saturated colour is inherent to the product. Coloured BGN fibre fractions could also be applied in some food applications, where besides providing nutritional and technological benefits it could be used as a natural colorant.

Polyphenolic composition of BGN fibres

Table 3.2 details the polyphenolic content of BGN insoluble and soluble fibres. Condensed tannins [CT] were detected in all BGNIF varieties ranging from 0.014 – 0.160 $mg.g^{-1}$. Brown (0.160 ± 0.007 $mg.g^{-1}$) BGNIF with the highest CT content corresponding to CT detected in cherry, plum and strawberry IDF (ranging from 0.11 – 0.12 $mg.g^{-1}$) (Goñi *et al.*, 2009),

Table 3.2 Phenolic content of Bambara groundnut insoluble and soluble dietary fibre[1, 2]

	Condensed tannins (mg.g^{-1})	Polyphenols (mg.g^{-1} GAE)
BGNIF		
BLE	0.014 ± 0.002^{a}	6.17 ± 1.00^{a}
RED	0.100 ± 0.005^{b}	13.81 ± 4.20^{b}
BRN	0.160 ± 0.007^{c}	15.56 ± 2.24^{b}
BRE	0.020 ± 0.000^{a}	6.14 ± 0.17^{a}
BGNSF		
BLE	nd	55.90 ± 1.63^{a}
RED	nd	52.95 ± 1.80^{a}
BRN	nd	46.27 ± 1.98^{b}
BRE	nd	45.42 ± 0.64^{b}

[1] Values are Mean ± Standard deviation. Means within a column followed by the same letter (under the same heading) are not significantly [p > 0.05] different.
[2] GAE: gallic acid equivalents; BGNIF: Bambara groundnut insoluble dietary fibre; BGNSF: Bambara groundnut soluble dietary fibre; BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye; nd: not determined.

differed significantly [$p \leq 0.05$] from the other varieties. The polyphenol [PP] content ranged from 6.14 – 15.56 $mg.g^{-1}$ GAE in BGNIF varieties. Brown-eye (6.14 ± 0.17 $mg.g^{-1}$ GAE) and black-eye (6.17 ± 1.00 $mg.g^{-1}$ GAE) BGNIF were significantly [$p \leq 0.05$] lower in PPs compared to higher levels found in the red (13.81 ± 4.20 $mg.g^{-1}$ GAE) and brown (15.56 ± 2.24 $mg.g^{-1}$ GAE) varieties. Considerably higher amounts of PPs were found in BGNSF varieties ranging from 45.42 – 55.90 $mg.g^{-1}$ GAE. Black-eye (55.90 ± 1.63 $mg.g^{-1}$ GAE) and red (52.95 ± 1.80 $mg.g^{-1}$ GAE) BGNSF with highest PP content differed significantly [$p \leq 0.05$] from the brown and brown-eye varieties.

Polyphenols, one of the most important groups of plant substances, are ubiquitous in the plant kingdom and consequently forms an important part in the diets of both animals and humans. Condensed tannins are insoluble polyphenols with high molecular weights, and are associated with the insoluble dietary fibre fractions. Also known as proanthocyanidins, these high molecular weight polymers are considered as anti-nutritional compounds due to the formation of complexes with cell wall polysaccharides or proteins, which hinders the digestibility of these nutrients in the intestinal tract (Saura-Calixto & Bravo, 2001). The role of polyphenols in dietary fibre includes that of profoundly affecting the physicochemical characteristics of fibres and determining their physiological properties in the human body. In the small intestine polyphenols are not absorbed and alongside dietary fibres they enter the colon where bacterial microflora uses them as fermentable substrates (Goñi *et al.*, 2009). As bioactive compounds some polyphenols provide high antioxidant activity to dietary fibres, which would allow the use of fibres as ingredients in the stabilisation of fatty foods, contributing to oxidative stability and an increase in shelf life (Elleuch *et al.*, 2011). The condensed tannins content of all BGNIF varieties were fairly low, with black-eye and brown-eye BGNIF having the lowest CT content. This indicates that CT forms a minor part of the polyphenols present in the BGN fibres, thus positively highlighting the potential of BGNIF, and more so BGNSF as ingredients with antioxidant potential. Little information is available on the polyphenolic content of commercial legume fibres. Agboola *et al.* (2010) found the total phenolic content in three commercial pea fibres ranging from 0.006 – 0.024 $mmol.g^{-1}$ GAE, where it was noted that the pea fibre with the highest phenolic content (0.024 $mmol.g^{-1}$ GAE) is a combination of pea fibre and starch fractions. The two high-fibre pea fractions were characterised with a lower total phenolic content. BGN fibre fractions could thus potentially offer more beneficial antioxidant properties as compared to commercial pea fibres.

Monomer composition of BGN fibres

The sugar composition of BGN fibres are presented in Table 3.3. The major neutral sugars found in the insoluble fibre varieties were the co-eluted arabinose and galactose (31.0 – 37.1%), xylose (16.5 – 27.3%) and mannose (14.5 – 22.2%). No significant [$p > 0.05$] difference was found for the arabinose/galactose amounts between BGNIF varieties.

Table 3.3 Sugar composition (% dry matter) of Bambara groundnut insoluble and soluble dietary fibres[1, 2]

	Rhamnose	Fucose	Ara/ Gal	Xylose	Mannose	Glucose	Fructose	Uronic acids
BGNIF								
BLE	nd	2.6 ± 1.0^{a}	37.1 ± 9.3^{a}	16.5 ± 1.2^{a}	14.5 ± 3.1^{a}	2.9 ± 0.3^{ac}	2.4 ± 0.6^{a}	24.0 ± 4.2^{a}
RED	nd	2.9 ± 1.1^{a}	30.4 ± 8.5^{a}	25.5 ± 2.8^{b}	20.4 ± 5.1^{ab}	5.5 ± 0.8^{b}	1.8 ± 0.1^{a}	13.5 ± 1.4^{b}
BRN	nd	1.9 ± 0.4^{a}	33.1 ± 7.5^{a}	22.9 ± 4.9^{b}	22.2 ± 3.1^{b}	2.5 ± 0.4^{a}	2.7 ± 0.4^{a}	14.8 ± 1.2^{b}
BRE	nd	2.0 ± 1.3^{a}	31.0 ± 3.9^{a}	27.3 ± 2.4^{b}	17.4 ± 2.5^{ab}	3.5 ± 0.3^{c}	2.5 ± 0.5^{a}	16.3 ± 1.2^{b}
BGNSF								
BLE	nd	4.8 ± 0.6^{a}	7.9 ± 0.2^{a}	54.5 ± 2.4^{a}	24.9 ± 2.0^{a}	3.0 ± 0.5^{a}	0.9 ± 0.5^{a}	4.0 ± 0.1^{a}
RED	nd	3.1 ± 1.0^{b}	10.9 ± 0.1^{a}	41.1 ± 4.6^{b}	33.0 ± 3.1^{b}	2.8 ± 0.6^{a}	3.6 ± 0.9^{b}	5.6 ± 0.0^{a}
BRN	nd	1.6 ± 0.3^{c}	8.4 ± 2.0^{a}	42.7 ± 6.8^{b}	34.3 ± 4.4^{b}	7.4 ± 1.4^{b}	1.3 ± 0.6^{a}	4.3 ± 1.0^{a}
BRE	nd	1.7 ± 0.3^{c}	10.8 ± 3.8^{a}	38.5 ± 2.3^{b}	37.5 ± 5.8^{b}	4.8 ± 0.4^{c}	1.3 ± 0.4^{a}	5.5 ± 1.9^{a}

[1] Results expressed as percentage of total sugars. Values are Mean ± Standard deviation. Means within a column followed by the same letter (under the same heading) are not significantly [$p > 0.05$] different.
[2] BGNIF: Bambara groundnut insoluble dietary fibre; BGNSF: Bambara groundnut soluble dietary fibre; BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye; Ara: arabinose; Gal: galactose; nd: not detected.

Dalgetty & Baik (2003) found consistent co-elution of arabinose and rhamnose as the major proportion in insoluble pea, lentil and chickpea cotyledon fibres. The authors ascribed this association to the findings by Pfoertner and Fischer (2001) in which it was noted that fibres from lupine cotyledons contained a rhamnogalacturonan backbone with side chains of arabinose and galactose. Ajila & Prasada Rao (2013) reported that high proportions of arabinose and galactose could be attributed to neutral arabinogalactan type polysaccharides linked to galacturonic acid residues. Since moderate amounts of uronic acids (13.5 – 24.0%) were also associated with the BGNIF varieties, the arabinogalactan polysaccharides could be indicative of the type of polysaccharides present in the BGNIFs. The xylose content was significantly [$p \leq 0.05$] higher in red (25.5 ± 2.8%), brown (22.9 ± 4.9%) and brown-eye (27.3 ± 2.4%) BGNIF. Dalgetty & Baik (2003) reported a significant amount of xylose (19.8 – 20.9%) associated with insoluble legume fibres, and Redondo-Cuenca *et al.* (2006) also reported the presence of xylose in yellow and green soybeans in proportions of 12 – 14% (yellow soybeans) and 19 – 21% (green soybeans). The presence of xylose and arabinose is indicative of arabinoxylans which forms part of hemicellulosic constituents (Dalgetty & Baik, 2003). Mannose content in BGNIF varieties were fairly high compared to findings by Redondo-Cuenca *et al.* (2006) where mannose content ranged from 2 – 4% (yellow soybeans) and 1 – 2% (green soybeans), and findings by Dalgetty & Baik (2003) which indicated the absence of mannose from pea, lentil and chickpea cotyledon fibres. Minor neutral sugar constituents in all BGNIF variants were fucose (1.9 – 2.9%), fructose (1.8 – 2.7%) and glucose (2.5 – 5.5%), whereas rhamnose was not detected. The glucose proportions were quite low compared to findings for other legume fibres, where glucose constitutes the major portion indicative of cellulose as predominant polysaccharide (Redondo-Cuenca *et al.*, 2006). The sugar composition of BGNIF varieties is thus indicative of hemicelluloses as a major polysaccharide.

The major neutral sugars present in BGNSF varieties were xylose (38.5 – 54.5%) and mannose (24.9 – 37.5%), which compared to the BGNIF varieties, constitutes a higher proportion in the soluble fibres. The xylose content of black-eye (54.5 ± 2.4%) BGNSF was the highest and differed significantly [$p \leq 0.05$] from the other BGNSF's. Comparably, lower xylose content was observed by Dalgetty & Baik (2003) in chickpea soluble cotyledon fibre (32%), and Redondo-Cuenca *et al.* (2006) found xylose content in a low range of 1 – 4% (yellow soybeans) and 5% (green soybeans). The arabinose/galactose constituents ranged from 7.9 – 10.9% [$p > 0.05$] in BGNSF varieties. The fairly high proportion of mannose and possibly galactose could be indicative of galactomannans presence or they could form part of pectins which represents the major polysaccharide of soluble fibres. (Lecumberri *et al.*, 2007). Well-known galactomannans are the locust bean gum extracted from the carob tree and guar gum extracted from the guar plant. Both gums are characterised by linear mannose chains with 1–4 β-ᴅ glycosidic bonds, and side chains of 1,6-bound galactose units

which are arranged differently on the mannose units (every two and four mannose units for guar gum and locust bean gum respectively). These structures lend cold solubility to guar gum and hot solubility to locust bean gum. Both guar gum and locust bean gum are commonly used in fruit juice beverages where they impart viscosity and mouthfeel (Fallourd & Viscione, 2009). Hence, the high mannose content and galactose presence allows for classification of BGNSF's in the same group as guar and locust bean gums. Fucose (1.6 – 4.8%), glucose (2.7 – 7.4%) and fructose (0.9 – 3.6%) were also present in BGNSF's in small proportions, and similar to the BGNIF fractions, rhamnose was not detected. The uronic acids in BGNSF ranged from 4.0 – 5.6%, proportions which are considerably lower compared to amounts present in the insoluble BGN varieties. Redondo-Cuenca *et al.* (2006) reported high uronic acid content of 31 – 38% for yellow soybeans and 41 – 50% for green soybeans, whereas Dalgetty & Baik (2003) reported minute proportions of uronic acids (0.2 – 1.3%) for soluble pea, lentil and chickpea cotyledon fibres.

The monomeric composition of the BGN fibres was in agreement to the monomers primarily found in legumes (Mongeau & Brooks, 2003b). The characterisation of the sugar composition in legume fibres is important to establish their functional properties (Tosh & Yada, 2010); this is highlighted in the BGNSFs where the possible presence of galactomannans indicates a thickening function of the soluble fibres which could prove valuable in the formulation of beverages.

3.3.3 Functional properties of BGN fibres

The functional properties of BGN fibre fractions are presented in Table 3.4; and include viscosity, hydration (swelling capacity, water retention capacity) and fat absorption capacity of the BGNIF's, and fat absorption capacity of the BGNSFs.

Swelling capacity ranged from 6.37 – 7.72 $ml.g^{-1}$. The swelling capacity of brown-eye (7.72 ± 0.49 $ml.g^{-1}$) BGNIF was the highest and differed significantly [$p \leq 0.05$] from the other three varieties. These results compare favourably to those reported by Dalgetty & Baik (2003) with the lowest swelling capacity for chickpea insoluble cotyledon fibre (4.28 $ml.g^{-1}$) and the highest for lentil insoluble cotyledon fibre (8.04 $ml.g^{-1}$). Swelling capacity of oat and bamboo fibres are reported as 4.98 and 5.69 $ml.g^{-1}$ respectively (Rosell *et al.*, 2009), and that of commercial pea fibres ranges from 6.4 – 8.3 $ml.g^{-1}$ (Wang & Toews, 2011). Compared to the swelling capacity of commercial fibres, the BGNIF varieties exhibited fairly high swelling capacity. Fibres with a high swelling capacity (i.e. ability to increase in bulk) contribute to a controlled energy balance in the diet, which are of importance in weight management as it influences satiety levels (Buttriss & Stokes, 2008). Swelling capacity of black-eye (6.83 ± 0.29 $ml.g^{-1}$) BGNIF was similar to that of apple fibre (6.89 ± 0.11 $ml.g^{-1}$) (Rosell *et al.*, 2009).

Table 3.4 Functional properties of Bambara groundnut insoluble and soluble dietary fibre[1, 2]

	SC (ml.g^{-1})	WRC (g water.g^{-1} dw)	FA (g oil.g^{-1} dw)
BGNIF			
BLE	6.83 ± 0.29^{a}	2.01 ± 0.05^{a}	1.49 ± 0.04^{a}
RED	6.50 ± 0.50^{a}	1.63 ± 0.43^{a}	1.38 ± 0.01^{b}
BRN	6.37 ± 0.23^{a}	1.69 ± 0.06^{a}	1.52 ± 0.04^{a}
BRE	7.72 ± 0.49^{b}	1.65 ± 0.07^{a}	1.49 ± 0.08^{a}
BGNSF			
BLE	Nd	nd	4.04 ± 0.12^{a}
RED	Nd	nd	4.46 ± 0.08^{b}
BRN	Nd	nd	4.55 ± 0.05^{b}
BRE	Nd	nd	4.42 ± 0.13^{b}

[1] Values are Mean ± Standard deviation. Means within a column followed by the same letter (under the same heading) are not significantly [p > 0.05] different.
[2] SC: swelling capacity, WRC: water retention capacity, FA: fat absorption, dw: dry weight; BGNIF: Bambara groundnut insoluble dietary fibre; BGNSF: Bambara groundnut soluble dietary fibre; BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye; nd: not determined.

Water retention capacity ranged from 1.63 – 2.01 g water.g^{-1} dry weight. Black-eye BGNIF had the highest water retention capacity (2.01 ± 0.05 g water.g^{-1} dry weight) whilst the red BGNIF had the lowest WRC (1.63 ± 0.43 g water.g^{-1} dry weight), with no significant [$p > 0.05$] difference between the WRC of the four BGNIF's. The WRC of BGNIFs are fairly low when compared to WRC of commercial pea fibres (3.7 – 4.5 g water.g^{-1} dry weight) as reported by Wang & Toews (2011). However, a fibre with reduced WRC could be of benefit in foods which are traditionally used for fibre enrichment (i.e. bread products), as it provides easy incorporation of the fibre into the food product (Tosh & Yada, 2010). It is thus shown that hydration properties of dietary fibres are not only responsible for determining the outcome of dietary fibres in the digestive tract, but they also play a role in the physiological effects of dietary fibre and influence product yield, ingredients functionality and shelf stability of many food products (Guillon & Champ, 2000; Rosell *et al.*, 2009).

The fat absorption capacity of BGNIFs ranged from 1.38 – 1.52 g oil.g^{-1} dry weight. Fat absorption was the lowest for red (1.38 ± 0.01 g oil.g^{-1} dry weight) BGNIF which differed significantly [$p \leq 0.05$] from the other three varieties. Comparably the fat absorption capacity of BGNSFs was higher, ranging from 4.04 – 4.55 g oil.g^{-1} dry weight with black-eye BGNSF having significantly [$p \leq 0.05$] lower fat absorption. Dalgetty & Baik (2003) reported FA for insoluble cotyledon pea, lentil and chickpea fibres and soluble cotyledon fibres ranging from 4.01 – 6.93 g oil.g^{-1} dry weight and 0.89 – 1.15 g oil.g^{-1} dry weight respectively, amounts which are considerably different to those obtained for the BGN fibres. These differences could be attributed to the differences in methodology used (i.e. longer vortex times over a longer time period and weighing of excess oil after centrifugation as opposed to the residue) and also the fat absorption capability of the cotyledon IDF and SDF fractions which might not behave in a similar manner to fibres from the whole seed (i.e. hull and cotyledon). Fat absorption for commercial pea fibres (1.23 – 1.33 g oil.g^{-1} dry weight) as reported by Wang & Toews (2011) was lower than those obtained from BGN fibres. This is indicative that fat absorption of both soluble and insoluble BGN fibres are comparable and in the case of BGNSF varieties, superior to that of commercial pea fibres. Fat absorption of fibres are important in food applications where dietary fibres with the ability to retain oil are important in the prevention of fat losses upon cooking (Anderson & Berry, 2001), and in providing stability in high-fat products and emulsions (Tiwari & Cummins, 2011).

Apparent viscosity was determined at BGNIF concentrations of 1%, 3%, 5% and 7%. At a shear rate of 793.7.sec^{-1}, viscosities increased at increasing concentrations for all BGNIF varieties. The highest apparent viscosities were observed for the brown BGNIF and the lowest for the black-eye BGNIF (Figure 3.5). Brown BGNIF had the highest significant [$p \leq 0.05$] viscosity at 5% and 7% w/v, and black-eye BGNIF the lowest significant [$p \leq 0.05$] viscosity at levels 1% and 7% w/v. Physiologically the role of viscous fibres includes that of prolonged transit time in the small intestine and thus increased absorption rates of nutrients,

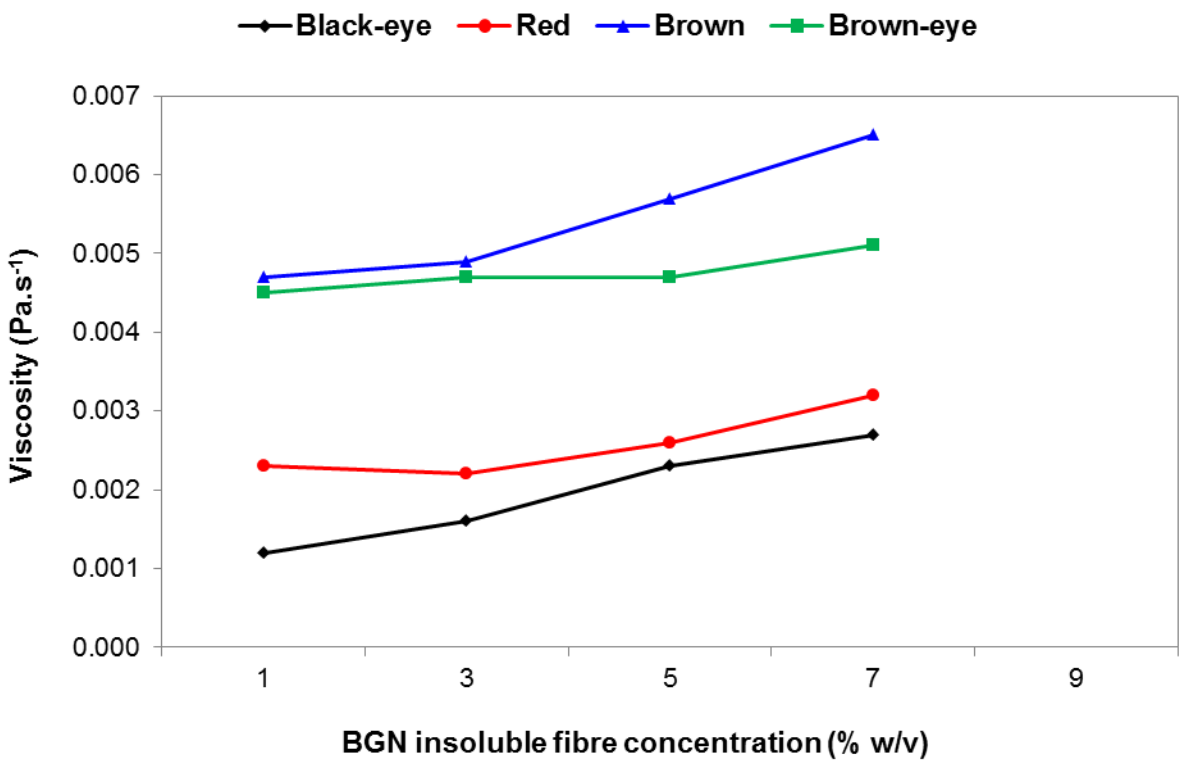


Figure 3.5 Effect of Bambara groundnut [BGN] insoluble dietary fibre concentration on viscosity (shear rate: 793.7/s; temperature: 25°C).

by increasing the digesta viscosity in the small intestine (Kristensen & Jensen, 2011). All BGNIFs were characterised by low viscosities. These fibres would ideally be used in non-viscous food systems and could be added to foods to enhance the nutritional content without negatively impacting textural characteristics.

3.3.4 Principal components

Principal components explaining variability in BGNIF physicochemical properties

The suitability of data reduction by PCA was established by several factors such as the high correlations between the variables (correlation matrix) and the significant [$p \leq 0.05$] Bartlett's test, as well as Kaiser-Meyer-Olkin measure (0.583) which was close to the recommended minimum of 0.6.

PCA with Varimax rotation (Table 3.5) was therefore applied to the BGNIF physicochemical and functional properties. Variation in the data could be explained by two components with eigenvalues greater than one. Component 1 accounted for 50.5% of variability, and represented the polyphenolics content, redness and yield of the BGNIF's. Component 2 accounted for 40.8% of variability, and represented the xylose content, yellowness and chroma of the BGNIFs. The cumulative variation of the two components amounted to 91.2%.

The score plot for component 1 and 2 is shown in Figure 3.6. The red and brown BGNIF's were grouped in close proximity with high (positive) values of component 1, whereas brown-eye BGNIF was located on the far lower (negative) end of component 1 with black-eye BGNIF situated between -1 and 0. Component 1 which represents the condensed tannins, polyphenols, redness and yield of the BGNIFs could thus be used to differentiate between the red/brown and brown-eye/black-eye BGNIFs. With respect to component 2, the brown-eye, red and brown BGNIFs were located positively (with one brown-eye replicate being an outlier located negatively to component 2) and were separated from black-eye BGNIF which were located between -2 and -1. The xylose content, yellowness and chroma of black-eye BGNIF could thus be used to differentiate it from the other varieties.

The colour of dietary fibres is a determinant for their inclusion in food applications, as it affects sensory characteristics (Tosh & Yada, 2010). These two components (condensed tannins, polyphenols, redness, BGNIF yield; and xylose content, yellowness and chroma respectively) identified in PCA could thus be used for prediction of possible end uses of the BGNIFs.

Table 3.5 Coefficient correlations between variables and components for physicochemical properties of BGNIFs[1]

Variable	Component	
	1	**2**
Polyphenolic content	0.937	0.143
Condensed tannins	0.870	0.286
Redness	0.868	0.450
Yield	0.865	0.449
Xylose %	0.743	-0.247
Yellowness	0.238	0.943
Chroma	0.310	0.628

[1] BGNIF: Bambara groundnut insoluble dietary fibre.

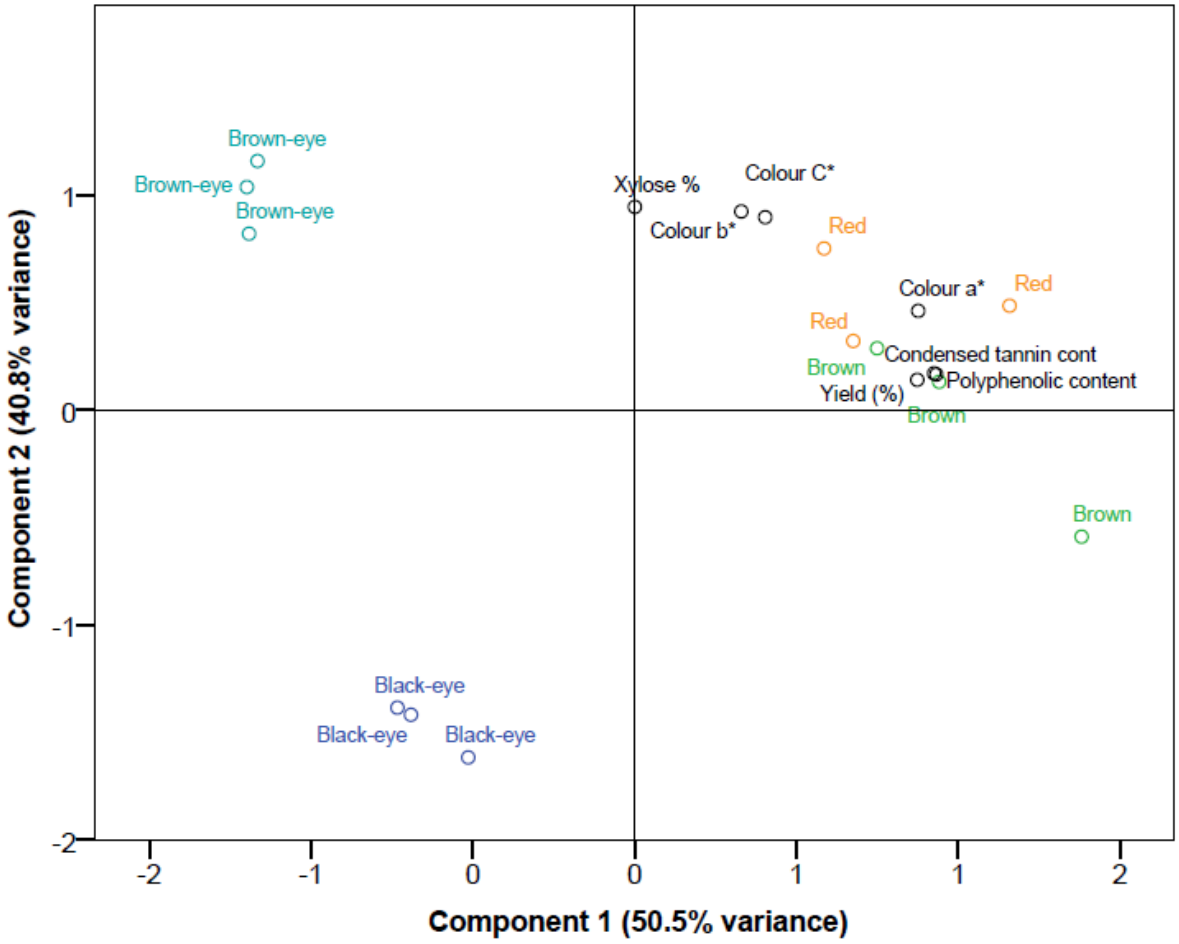


Figure 3.6 Score plot showing differentiation of Bambara groundnut insoluble dietary fibre varieties with respect to components 1 and 2.

Principal components explaining variability in BGNSF physicochemical and functional properties

The correlation matrix showed high correlations between variables; and although the Kaiser-Meyer-Olkin (0.457) measure was below the recommended minimum of 0.6, the Bartlett's test [$p \leq 0.05$] was significant which indicated that PCA might be suitable for data reduction. PCA with Varimax rotation (Table 3.6) was therefore carried out and applied to BGNSF physicochemical and functional properties.

The variability in the data could be explained by two components with eigenvalues greater than one. Variation (40.8%) was attributed to component 1 which represented yellowness, chroma and mannose content of BGNSFs, whilst 38.6% of variation was attributed to component 2 which represented redness, fat absorption and fructose content of BGNSFs. Both components accounted for a cumulative variation of 79.4%.

The score plot indicating the relationship of BGNSF varieties to the components is shown in Figure 3.7. Component 1 separates the red and brown-eye varieties (located positively) from the brown and black-eye varieties (located negatively). A distinction between these varieties can thus be made on some of their colour parameters and mannose content. Component 2 separates the brown and red varieties (located positively) from the black-eye and brown-eye varieties (located negatively), which indicates that the redness, fat absorption capacity and fructose content of these fibres can be used to differentiate between them. The colour of dietary fibres affects sensory characteristics and is therefore an important property to consider for their inclusion in different food applications. The sugar composition of dietary fibres is also important in establishing their functional properties (Tosh & Yada, 2010). These two components would therefore be adequate for prediction of possible end uses and functional properties of BGNSFs.

3.4 Conclusion

Bambara groundnut insoluble and soluble dietary fibres were successfully extracted from four varieties (black-eye, red, brown and brown-eye). The objective of isolating the soluble and insoluble fractions from BGN was thus attained. BGN fibres showed positive physicochemical and functional properties. The insoluble fibres were characterised by a yellowish-red colour, with black-eye BGNIF having the highest lightness which could prove beneficial for incorporation into bakery products. Similarly, all BGNSF varieties had characteristic high lightness with colour parameters indicating a light yellow colour. Yellowness for brown BGNIF and red BGNSF were comparable to that of commercial pea fibres. The bulk density for all BGN insoluble and soluble fibres was considerably higher compared to other pulse cotyledon fibres, which could prove beneficial in packaging cost considerations. Black-eye and red BGNSF had the highest polyphenol content, which

Table 3.6 Coefficient correlations between variables and components for physicochemical and functional properties of BGNSFs[1]

	Component	
Variable	**1**	**2**
Yellowness	0.956	0.046
Chroma	0.878	0.409
Mannose %	0.751	0.263
Redness	0.103	0.968
Fructose %	0.190	0.779
Fat absorption	0.390	0.732

[1] BGNSF: Bambara groundnut soluble dietary fibre.

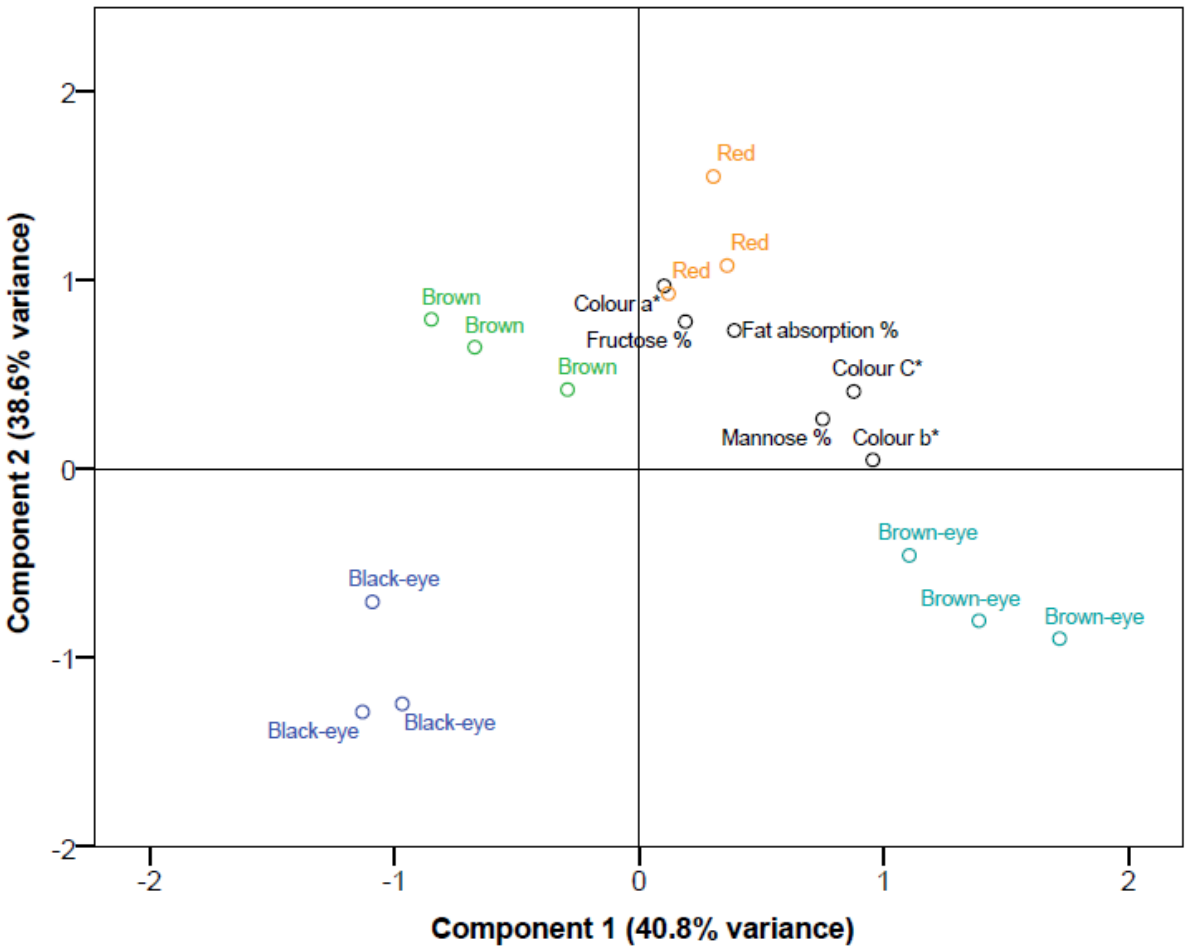


Figure 3.7 Score plot showing differentiation of Bambara groundnut soluble dietary fibre varieties with respect to components 1 and 2.

indicates the possible antioxidant activity of these fibres. The major neutral sugars in BGNIF varieties were the co-eluted arabinose/galactose, xylose and mannose. The proportion of these monomers is indicative of hemicellulosic polysaccharides. Xylose and mannose was also the major constituents of BGNSFs and together with galactose were indicative of galactomannans which forms part of pectic substances. All BGNIF varieties had a higher swelling capacity compared to commercial fibres, with black-eye BGNIF having a swelling capacity comparable to apple fibre. Fat absorption capacity of BGNIF and BGNSF varieties were higher compared to commercial pea fibres. The BGN fibres were characterised based on their functional and physicochemical properties, and potential food applications have been identified accordingly. The objective of characterisation of the soluble and insoluble BGN fibres was thus also accomplished. The hypothesis that the non-starch polysaccharides extracted from the four BGN varieties will differ from each other in their functionality in model systems is accepted, since some differences were observed between the BGN fibre varieties for hydration and fat absorption properties. It can be concluded that the four BGNIF and BGNSF varieties have the potential to be used as sustainable sources of insoluble and soluble fibre, proving to be comparable or superior to commercial fibre in many instances.

References

Abdul-Hamid, A. & Luan, Y.S. (2000). Functional properties of dietary fibre prepared from defatted rice bran. *Food Chemistry*, **68**, 15-19.

Adebowale, K.O., Afolabi, T.A. & Lawal, O.S. (2002). Isolation, chemical modification and physicochemical characterisation of bambara groundnut (*Voandzeia subterranean*) starch and flour. *Food Chemistry*, **78**, 305-311.

Agboola, S.O., Mofolasayo, O.A., Watts, B.M. & Aluko, R.E. (2010). Functional properties of yellow field pea (*Pisum* sativum L.) seed flours and the in vitro bioactive properties of their polyphenols. *Food Research International*, **43**, 582-588.

Ajila, C.M. & Prasada Rao, U.J.S. (2013). Mango peel dietary fibre: composition and associated bound phenolics. *Journal of Functional Foods*, **5**, 444-450.

Anderson, E.T. & Berry, B.W. (2001). Effects of inner pea fiber on fat retention and cooking yield in high fat ground beef. *Food Research International*, **34**, 689-694.

Anderson, J.W., Baird, P., Davis Jr., R.H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V. & Williams, C.L. (2009). Health benefits of dietary fibre. *Nutrition Reviews*, **67(4)**, 188-205.

AOAC (2005). Official Method **991.43**. *Official Methods of Analysis of AOAC INTERNATIONAL* 18th Edition. Gaithersburg, MD: AOAC International.

Benítez, V., Cantera, S., Aguilera, Y., Mollá, E., Esteban, R.M., Díaz, M.F. & Martín-Cabrejas, M.A. (2013). Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes. *Food Research International*, **50**, 64-69.

Buttriss, J.L. & Stokes, C.S. (2008). Dietary fibre and health: an overview. *Nutrition Bulletin*, **33**, 186-200.

Chawla, R. & Patil, G.R. (2010). Soluble dietary fibre. *Comprehensive Reviews in Food Science & Food Safety*, **9**, 178-196.

Dalgetty, D.D. & Baik, B. (2003). Isolation and characterization of cotyledon fibers from peas, lentils, and chickpeas. *Cereal Chemistry*, **80(3)**, 310-315.

Daou, C. & Zhang, H. (2011). Physico-chemical and antioxidant activities of dietary fiber derived from defatted rice bran. *Advance Journal of Food Science and Technology*, **3(5)**, 339-347.

De Vries, J.W. (2003). On defining dietary fibre. *Proceedings of the Nutrition Society*, **62**, 37-43.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*, **124**, 411-421.

Eltayeb, A.R.S.M., Ali, A.O., Abou-Arab, A.A. & Abu-Salem, F.M. (2011). Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut (*Vigna subterranea* (L.) Verdc.). *African Journal of Food Science*, **5(2)**, 82-90.

Englyst, K.N., Liu, S. & Englyst, H.N. (2007). Review: Nutritional characterisation and measurement of dietary carbohydrates. *European Journal of Clinical Nutrition*, **61**, S19-S39.

Fallourd, M.J. & Viscione, L. (2009). Ingredient selection for stabilisation and texture optimisation of functional beverages and the inclusion of dietary fibre. In: *Functional and Speciality Beverage Technology* (edited by P. Paquin). Pp. 12-13. Florida, USA: Woodhead Publishing Limited.

Goñi, I., Díaz-Rubio, M.E., Pérez-Jiménez J. & Saura-Calixto, F. (2009). Towards an updated methodology for measurement of dietary fiber, including associated polyphenols, in food and beverages. *Food Research International*, **42**, 840-846.

Guillon, F. & Champ, M. (2000). Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International*, **33**, 233-245.

Hung, C. & Yen, G. (2002). Antioxidant activity of phenolic compounds isolated from *Mesona procumbens* Hemsl. *Journal of Agricultural and Food Chemistry*, **50**, 2993-2997.

Jideani, V.A. & Diedericks, C.F. (2014). Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*. In: *Antioxidant-Antidiabetic Agents and Human Health* (edited by O. Oguntibeju). Pp. 187-207. Rijeka, Croatia: Intech Publishers.

Jiménez-Escrig, A. & Sánchez-Muniz, F.J. (2000). Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutrition Research*, **20**, 585-598..

Kristensen, M. & Jensen, M.G. (2011). Dietary fibres in the regulation of appetite and food intake. Importance of viscosity. *Appetite*, **56**, 65-70.

Lan, G., Chen, H., Chen, S. & Tian, J. (2012). Chemical composition and physicochemical properties of dietary fiber from *Polygonatum odoratum* as affected by different processing methods. *Food Research International*, **49**, 406-410.

Lecumberri, E., Mateos, R., Izquierdo-Pulido, M., Rupérez, P., Goya, L. & Bravo, L. (2007). Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.). *Food Chemistry*, **104**, 948-954.

Mañas, E., Bravo, L. & Saura-Calixto, F. (1994). Sources of error in dietary fibre analysis. *Food Chemistry*, **50**, 331-342.

Mann, J.I. & Cummings, J.H. (2009). Possible implications for health of the different definitions of dietary fibre. *Nutrition, Metabolism and Cardiovascular Diseases*, **19**, 226-229.

Mongeau, R. & Brooks, S.P.J. (2003a). Dietary fibre determination. In: *Encyclopedia of Food Sciences and Nutrition*, 2nd Edition (edited by B. Caballero, L. Trugo & P.M. Finglas). Pp. 1823-1833. New York: Elsevier Science Ltd.

Mongeau, R. & Brooks, S.P.J. (2003b). Dietary fiber properties and sources. In: *Encyclopedia of Food Sciences and Nutrition*, 2nd Edition (edited by B. Caballero, L. Trugo & P.M. Finglas). Pp. 1813-1823. New York: Elsevier Science Ltd.

Parrott, M.E. & Thrall, B.E. (1978). Functional properties of various fibers: physical properties. *Journal of Food Science*, **43**, 759-764 (As cited by Dalgetty & Baik, 2003; Tiwari & Cummins, 2011).

Pfoertner, H.N. & Fischer, J. (2001). Dietary fibres of lupins and other grain legumes. In: *Advanced Dietary Fibre Technology* (edited by B.V. McCleary & L. Prosky). Pp. 361-366. UK: Blackwell Science Ltd.

Redondo-Cuenca, A., Villanueva-Suárez, M.J., Rodríguez-Sevilla, M.D. & Mateos-Aparicio, I. (2006). Chemical composition and dietary fibre of yellow and green commercial soybeans (Glycine max). *Food Chemistry*, **101**, 1216-1222.

Rodríguez, R., Jiménez, A., Fernández-Bolaños, J., Guillén, R. & Heredia, A. (2006). Dietary fibre from vegetable products as source of functional ingredients. *Trends in Food Science & Technology*, **17**, 3-15.

Roehrig, K.L. (1988). The physiological effects of dietary fibre. *Food Hydrocolloids*, **2**, 1-18.

Rosell, C.M., Santos, E. & Collar, C. (2009). Physico-chemical properties of commercial fibres from different sources: A comparative approach. *Food Research International*, **42**, 176-184.

Saura-Calixto, F.D. & Bravo, L. (2001). Dietary fiber-associated compounds: Chemistry, analysis, and nutritional effects of polyphenols. In: *Handbook of Dietary Fiber* (edited by S.S. Cho & M.L. Dreher). New York: Marcel Dekker, Inc.

Schaafsma, G. (2004). Health claims, options for dietary fibre. In: *Dietary Fibre* (edited by J.M. van der Kamp, N.G. Asp, J. Miller & G. Schaafsma). Pp. 27-38. The Netherlands, Wageningen Academic Publishers (As cited by Rodríquez *et al.*, 2006).

Tiwari, U. & Cummins, E. (2011). Functional and physicochemical properties of legume fibers. In: *Pulse Foods: Processing, Quality and Nutraceutical Applications* (edited by B.K. Tiwari, A. Gowen & B. McKenna). p. 121-147. London, UK: Elsevier Inc.

Tosh, S.M. & Yada, S. (2010). Review – Dietary fibres in pulse seeds and fractions: Characterisation, functional attributes, and applications. *Food Research International*, **43**, 450-460.

Turowski, M., Deshmukh, B., Harfmann, R., Conklin, J. & Lynch, S. (2007). A method for determination of soluble dietary fiber in methylcellulose and hydroxypropyl methylcellulose food gums. *Journal of Food Composition and Analysis*, **20**, 420-429.

Wang, N. & Toews, R. (2011). Certain physicochemical and functional properties of fibre fractions from pulses. *Food Research International*, **44**, 2515-2523.

CHAPTER FOUR

FUNCTIONALITY OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* (L.) VERDC.) INSOLUBLE DIETARY FIBRE IN WHITE BREAD

Abstract

Brown [BRN] bambara groundnut insoluble dietary fibre [BGNIF] was incorporated into a white bread formulation following a IV optimal mixture design to determine the optimum bread formulation. Three mixture components constrained at lower and upper limits (water: 57 – 60%, yeast: 2.3 – 5.3%, BGNIF: 7 – 10%) were evaluated for their effects on responses of specific loaf volume, gumminess, chewiness and resilience of the loaves. The linear model was found adequate to navigate the design space, and numerical optimisation established the best optimal formulation with desirability of 0.778 at 59.5% water, 4.3% yeast and 8.5% BGNIF. Bread enriched with four varieties of BGNIF (black-eye [BLE], red [RED], brown [BRN] and brown-eye [BRE]) were prepared according to the optimal formulation, and evaluated for some physicochemical properties. Crumb structure analysis indicated BGNIF breads had a higher number of pores compared to the control bread. The highest number of pores for both control and BGNIF enriched breads were concentrated in two categories, pores with area < 5.00 mm^2 and pores with area 5.00 – 24.99 mm^2. Pores from all loaves were non-circular in shape, and circularity differed significantly [$p \leq 0.05$] between BLE (0.25 ± 0.13) and RED (0.28 ± 0.15) BGNIF bread. Roundness of the pores confirmed their non-circular shape and no significant [$p > 0.05$] difference was found in roundness of the control (0.52 ± 0.20) bread pores and BRE (0.54 ± 0.18) bread pores. Chemical analysis included moisture, total dietary fibre [TDF] and polyphenol content of the loaves. The TDF content of all BGNIF loaves could be positively highlighted, as all varieties resulted in significantly [$p \leq 0.05$] higher TDF content ranging from 7.14 ± 0.24% to 8.33 ± 0.03%, compared to 4.96 ± 0.91% of the control loaf. Trace amounts of condensed tannins [CT] were found in all loaves with no significant [$p > 0.05$] difference found in CT content of control (0.0003 ± 0.0001 $mg.g^{-1}$) and BRE (0.0004 ± 0.0001 $mg.g^{-1}$) loaves. Polyphenols were significantly [$p \leq 0.05$] highest in BRN (5.06 ± 0.40 $mg.g^{-1}$ gallic acid equivalents) BGNIF bread. Control bread had a specific loaf volume of 4.16 ± 0.05 $ml.g^{-1}$, comparably BGNIF varieties significantly [$p \leq 0.05$] decreased specific loaf volume ranging from 3.33 – 3.85 $ml.g^{-1}$. Texture parameters evaluated were firmness, springiness, gumminess, resilience and chewiness. Favourable results were obtained for some parameters, but more so for firmness/hardness of the BGNIF enriched bread which had softer crumbs (ranging from 4.13 ± 0.74 to 6.75 ± 0.84 N) compared to the control (9.69 ± 0.19 N) bread. Crumb colour difference from the control bread was significantly [$p \leq 0.05$] lowest for BRE (3.43 ± 0.20) BGNIF bread whilst crust colour difference was significantly [$p \leq 0.05$] lowest for BRN (1.72 ± 0.42) and BRE (2.44 ± 0.78) loaves. Variability in bread physicochemical properties could be differentiated by three

principal components. All bread loaves were sensorially acceptable based on a rating of >3 for all the parameters (appearance, crust and crumb colour, aroma, taste, texture and overall acceptability) evaluated. Panellists could not differentiate [$p > 0.05$] between crust colour and texture of control and BGNIF enriched loaves. Panellists did perceive significant [$p \leq 0.05$] difference in aroma and taste of control and BGNIF loaves. Regression analysis revealed taste rating was impacted by BGNIF, gender and international status, whilst aroma rating was influenced by BGNIF, race and international status. When applying categorical principal component analysis [CATPCA] to the taste parameter, the percentage of variance for dimension 1 was 36.1% and for dimension 2 it was 28.2% with BLE and BRE BGNIF breads located close to "like very much" and "like moderately". For aroma the percentage of variance was 34.9% for dimension 1 and 33.1% for dimension 2, with BRE and control breads positioned close to "like moderately" and "like very much" as preferred by black international students. Bread enriched with BGNIF varieties revealed positive physicochemical properties with high consumer acceptability for all varieties, and especially for BRE and BLE breads.

4.1 Introduction

Dietary fibre is considered to be amongst the important ingredients to be included in the diet; this is as a consequence of the distinct physiological functionalities and numerous health benefits associated with an increased dietary fibre intake, as well as the technological properties dietary fibre imparts when incorporated into foods (Schaafsma, 2004; Elleuch *et al.*, 2011). The increased consumption of dietary fibre has been associated with the decreasing risk of the development of several diseases. This important role of dietary fibre is mainly related to cardiovascular health, the prevention and management of diabetes, obesity management, gastrointestinal function and health as well as the optimal function of the immune system (Tharanathan & Mahadevamma, 2003; Anderson *et al.*, 2009). Today, dietary fibre-based interventions are then also of great interest to researchers as a means of controlling obesity as well as reducing the risk of type II diabetes and cardiovascular disease (Brownlee, 2011). The physiological functionalities exerted by dietary fibres is influenced by their physicochemical properties such as viscosity, water holding capacity, fermentation, bulk and bile acid binding; all of which affects the small and large intestine differently (Schneeman, 1998; Blackwood *et al.*, 2000; Tharanathan & Mahadevamma, 2003). Insoluble dietary fibres are noted for their effect in the large intestine and support of intestinal microflora growth (Blackwood *et al.*, 2000; Tosh & Yada, 2010). Apart from their influence on physiological functionality, the physicochemical properties of dietary fibres have important technological effects when incorporated into food products such as gel-forming capacity, texturising and stabilising effects (Elleuch *et al.*, 2011).

As a result of the importance dietary fibre has in the diet, the market for products and ingredients rich in dietary fibre has grown largely and a trend has been observed to obtain new dietary fibre food sources for use in the food industry (Chau & Huang, 2003). Pulses, also referred to as legumes, are plant species of the family Leguminosae and are classified after cereal grains as the second most important food source in the world (Tiwari *et al.*, 2011). Recognised for their significant potential health benefits, the fractionation of pulses into major constituents can be explored to increase their use in food and beverage products (Tiwari *et al.*, 2011; Wang & Toews, 2011). Fibre fractions, one of the major pulse fractions, are commercially available in the form of pea hull and cotyledon fibres; however fibre fractions from other pulse sources are scarce (Wang & Toews, 2011). As determined by Wang & Toews (2011), pulse fibres have superior functional properties and this leads to a great potential for their use in various food applications. In their study, Wang & Toews (2011) investigated pulse fibre fractions from four of the eleven primary pulse classes as recognised by the FAO (Tiwari *et al.*, 2011), including several varieties of peas (Class: dry peas, *Pisum* spp.), lentils (Class: lentil, *Lens culinaris*), chickpeas (Class: chickpea, *Cicer arietinum*) and navy beans (Class: dry beans, *Phaseolus* spp.). Thus, other pulse classes remain to be investigated. From this knowledge, the gap in the market would be to expand the utilisation of pulse derived fibres, which would lead to enhanced human health as well as an increase in the market value of pulse crops (Wang & Toews, 2011; Tosh & Yada, 2010). One such pulse class, bambara groundnut [BGN] (*Vigna subterranea*), is an underutilised legume seed cultivated in Africa and could be a potential significant source of pulse fibre fractions (Eltayeb *et al.*, 2011). As reviewed in Jideani & Diedericks (2014), BGN have a rich nutritional profile with numerous health benefits, and by tapping into just one of these areas (such as the dietary fibre fractions) the economic status of BGN could be greatly changed.

In investigated or proposed food application studies for pulse fibre fractions, the general categories include fibre enrichment, nutrient fortification, fat binding and retention or texture modification (Tosh & Yada, 2010). Fibre incorporated into foods is known to change several characteristics of the end-products such as texture, consistency, rheological and sensorial properties (Guillon & Champ, 2000). These and several other technological properties imparted by dietary fibres, as well as their associated health benefits and physiological roles, make dietary fibres and fractions important as ingredients in the development of highly demanded value-added and functional foods (Abdul-Hamid & Luan, 2000; Dhingra *et al.*, 2012). Today, several food products are being enriched with dietary fibre; not only to increase the fibre content of the foods, but to provide additional benefits which are either marketing or technological-oriented (Endress & Fischer, 2001). The numerous food products to which dietary fibres have been added include bakery products, beverages, meat products, fish products, confectionary, frozen dairy and convenience products, dairy products, pastas, fruit preparations and soups (Endress & Fischer, 2001;

Elleuch *et al.*, 2011). Among these foods, bakery products are the most common foods traditionally selected for dietary fibre enrichment; due to the ability of fibres to retain water which prolongs freshness and consequently reduces economic losses (Elleuch *et al.*, 2011).

The aim of this study was therefore to incorporate bambara groundnut (*Vigna subterranea* (L.) Verdc.) insoluble dietary fibre into white bread formulation, with a view to highlight the potential of BGNIF varieties as new fibre sources for use in bread and potentially other food formulations.

4.2 Materials and Methods

The overview of the methodology employed in this chapter is shown in Figure 4.1. Briefly, four BGNIF varieties (black-eye, red, brown and brown-eye) were incorporated into an optimal white bread formulation following optimisation via an IV-optimal mixture design. The control white bread (prepared without BGNIF) and the BGNIF enriched bread loaves were subjected to several physicochemical analyses including specific loaf volume [SLV], crumb and crust colour measurement, moisture content, total dietary fibre [TDF] content, polyphenolic content, crumb grain characteristics and bread textural characteristics. Sensorial analysis indicating consumer acceptance of the bread was also performed. Detailed descriptions of the methodology are given in subsequent sections.

4.2.1 Source of materials

A commercial white bread flour (Sasko, South Africa), commercial compressed yeast (Anchor Yeast, South Africa), salt (Cape Town, South Africa), sugar (Cape Town, South Africa) and vegetable shortening were used in all bread formulations. Bambara groundnut insoluble dietary fibre [BGNIF] which was isolated from this project was used.

4.2.2 Bread-making procedure

The optimised straight-dough bread-making method, Approved Method 10-10.03 (AACC, 2000a) was used to prepare the bread samples. The bread formula based on a 100% flour basis contained 6% sugar, 1.5% salt, 3% vegetable shortening, 2 – 5.3% yeast, 57 – 60% water and 7 – 10% BGNIF. The actual amount of yeast, water and BGNIF were determined through a mixture design as detailed in section 4.2.3. Briefly the shortening was blended with the dry ingredients and placed in the dough mixer (Magimix Cuisine 5200, France), the liquids were added in the centre of the dry ingredients and the yeast (dissolved in water) added along the sides of the mixing bowl. Mixing was optimally performed for a total mixing time of 3 min. After mixing, the dough was transferred to a fermentation bowl and fermented

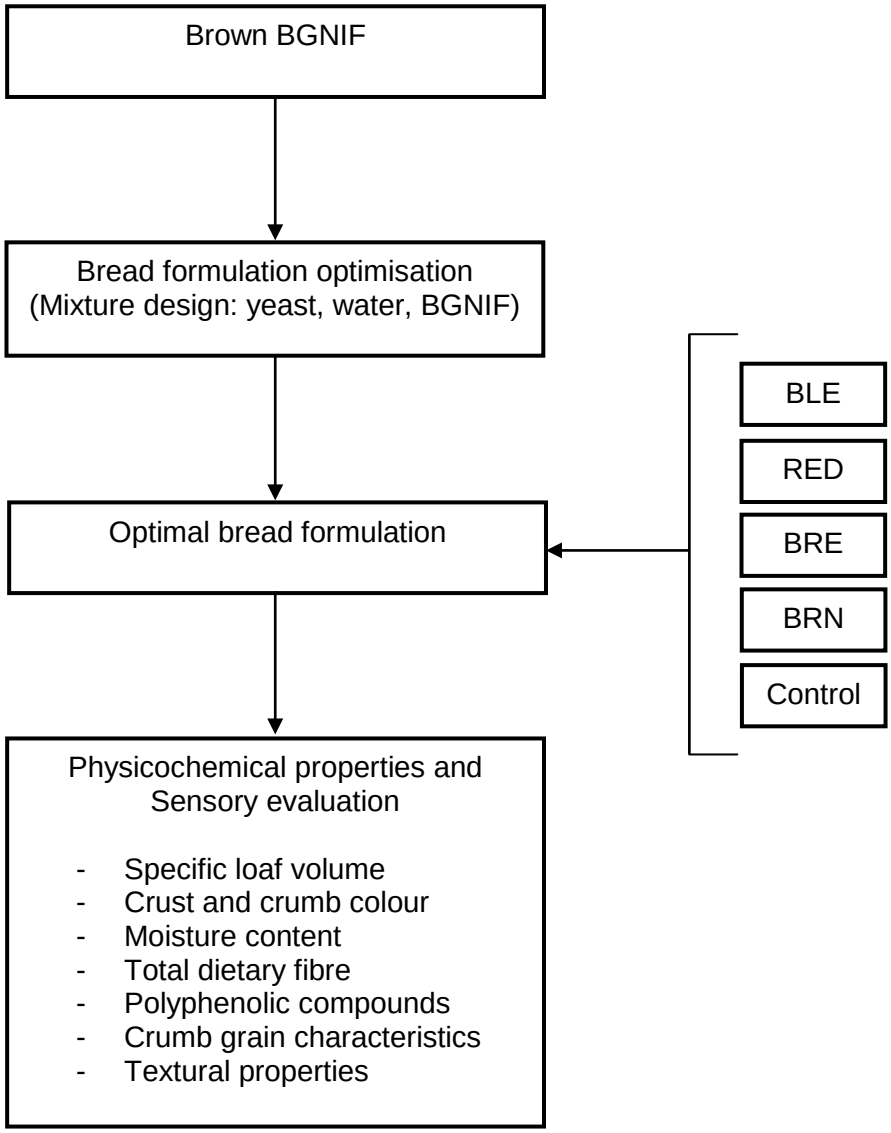


Figure 4.1 Methodology overview. BGNIF: Bambara groundnut insoluble dietary fibre, BLE: black-eye, RED: red, BRN: brown, BRE: brown-eye.

at 30°C for 90 min (punched twice) in a fermentation cabinet. Dough was then divided into 58 g pieces, hand-moulded and placed into baking pans, followed by proofing for 33 min. After proofing, the dough pieces were baked in a multi-deck oven at 215°C for 15 min. After cooling for 1 h at room temperature, all loaves were evaluated for weight and loaf volume, followed by storage for 24 h in airtight polypropylene bags for evaluation of bread physicochemical attributes.

4.2.3 Experimental design and data analysis for bread optimisation

To determine the optimum bread formulation, an IV-optimal mixture design was chosen as the desirable design type based on the number and constraints of the selected mixture components. The three mixture components were water (A), yeast (B) and BGNIF (C) from brown BGN. Each component was constrained with lower and upper limits; the three components in real scale amounted to 72.3% (57 – 60% for water, 2.3 – 5.3% for yeast and 7 – 10 % for BGNIF) per 100% flour basis. The design consisted of ten formulations (Table 4.1) consisting of three vertex points, two replicated vertices, three interior points and two edge points. The ten loaves were baked in randomised order following the procedure as specified in section 4.2.2 (page 80). The resulting bread loaves were measured for SLV and textural (including gumminess, resilience and chewiness) parameters as detailed in section 4.2.4 (page 84). The response variables were fitted to a linear mixture model (equation [1]).

$$Y = b_1X_1 + b_2X_2 + b_3X_3 \qquad [1]$$

Where, Y is the dependent variable to be predicted (SLV and texture parameters of the loaf), *b* the equation coefficients (b_1, b_2 and b_3 referring to coefficients of water, yeast and BGNIF respectively) and *X* the component proportions or independent variables (X_1, X_2 and X_3 referring to water, yeast and BGNIF respectively).

Analysis of variance (ANOVA) was used to determine the statistical significance for each response at 5% level. Goodness of the model fit was determined by the lack of fit F-value and adequate precision, and normality of the data was checked with residual plots. The Design-Expert (Stat-Ease Inc., USA, version 8.0.7, 2010) statistical software was used to determine the mixture experimental design, all data analysis, optimisation and all graphical plots. In Scheffé polynomial mixture models it is recommended that response surfaces are used to interpret the effects of mixture components, as opposed to the interpretation of linear coefficients which is dependent on the difference between the coefficients and not on their absolute magnitude (Stat-Ease, 2011). Two plots, Trace (Piepel) and three-dimensional (3D) surface plots were therefore used to interpret the effects of the mixture components on the

Table 4.1 Formulation sets as determined by three-component IV-optimal mixture design

	Proportion of components[1]		
Formulation	**Water (%)**	**Yeast (%)**	**BGNIF (%)**
1	60.0	2.3	10.0
2	60.0	2.3	10.0
3	60.0	5.3	7.0
4	57.0	5.3	10.0
5	59.5	4.8	8.0
6	57.0	5.3	10.0
7	58.5	5.3	8.5
8	59.0	4.3	9.0
9	58.5	3.8	10.0
10	60.0	3.8	8.5

[1]Component values indicated in actual scale; BGNIF: Bambara groundnut insoluble dietary fibre.

measured responses. The trace plot was used to compare the component effects on the design space, whilst the 3D plot clearly shows the response surface.

4.2.4 Production and physicochemical characteristics of optimal bread from different varieties of BGN fibre

Four varieties of BGNIF (black-eye, red, brown and brown-eye) were incorporated into the optimal bread formulation; whilst the control bread was prepared using all the ingredients as determined for the optimal bread, but excluding the BGNIF (as detailed in section 4.2.2, page 80). All the loaves were subjected to several physicochemical analyses including specific loaf volume, crust and crumb colour, moisture content, total dietary fibre, polyphenolic content, crumb grain and textural characteristics. Sensory evaluation was also performed to assess the consumer acceptance of BGNIF enriched bread.

Specific loaf volume

Breads were weighed 1 h after cooling, followed by determination of loaf volume by the seed displacement method (Cauvain & Young, 2006). Quinoa seeds were used as displacement medium. The volume of the container (500 ml) was determined by filling the container with seeds and noting the volume of seeds required to fill the container. The bread was placed inside the container and the seeds poured over the bread. The volume of seeds displaced represented the volume of the loaf (ml). SLV was calculated by dividing loaf volume by weight ($ml.g^{-1}$).

Crust and crumb colour

The crust and crumb colour of the loaves were evaluated with a spectrophotometer (Model CM-5, Konica Minolta Sensing, Japan) using the CIE-*L*a*b* and *L*C*h* colour space. Calibration of the instrument was performed with the white calibration plate followed by zero calibration. Three loaves were taken as replicates per formulation, and two slices per loaf were used as test pieces. The crust was carefully removed from the crumb. Crumb pieces were measured as is and the crust roughly ground before measurement. Samples were placed evenly in the provided petri-dish (diameter 30 mm) covering the bottom of the dish, to allow for reflectance measurement. Measurements for each sample were performed in triplicate at three different locations in the sample (one reading = average of three readings per rotated position), with the results recorded in *L** (lightness), *a** (chromaticity coordinate +*a** = red and –*a** = green), *b** (chromaticity coordinate +*b** = yellow and –*b** = blue), *C** (chroma) and *h* (hue angle 0° = red, 90° = yellow, 180° = green and 270° = blue) [SpectraMagic NX, version CM-S100w 2.03.0006, Konica Minolta, 2010]. The magnitude of

the colour difference between the control bread (standard) and enriched breads (sample) were calculated according to equation [2].

$$\Delta E^* = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})} \qquad [2]$$

Where, Δ*L** is the lightness difference ($L^*_{sample} - L^*_{standard}$), Δ*a** is the red/green difference ($a^*_{sample} - a^*_{standard}$) and Δ*b** is the yellow/blue difference ($b^*_{sample} - b^*_{standard}$) (Sahin & Sumnu, 2006a).

Crust and crumb moisture content

The moisture content of the loaves was determined by the Approved moisture air-oven Method 44-15.02 (AACC, 2000b).

Total dietary fibre content

TDF content in each loaf was determined by the Official Method 991.43 (AOAC, 2005).

Polyphenolic compounds

The condensed tannins and polyphenol content of each loaf were determined by absorbance measurement as is described under section 3.2.4 (page 50).

Crumb grain

The crumb grain refers to the size, number and distribution of cells in the bread crumb (Cauvain, 1999). Crumb grain analysis was performed by capturing images of the different bread slices with a digital camera. The digital images were imported from the camera in RGB standard format and saved in tiff format. Image analysis was performed using image analysis software (ImageJ, 1.47v, 2012). The pixel values of the imported images were converted from pixels to millimetre (control: 127.88 x 144.88 mm, black-eye: 124.83 x 134.47 mm, red: 131.94 x 141.07 mm, brown-eye: 123.82 x 135.74 mm and brown: 124.83 x 144.62) by using known length values. Thresholding was applied to the images as a segmentation technique, which allows the object of interest (in this case the pores or cells in the bread crumb) to be extracted from the background by transforming the foreground pixels to black and the background pixels to white (Aguilera & Germain, 2007). Crumb grain measurements were performed on the transformed images giving information on the number of pores present in the bread crumbs, pore size distributions, feret angle, aspect ratio, circularity and roundness of the pores.

Textural characteristics

Texture profile analyses (TPA) was performed on the loaves after cooling for 24 h by using an Instron Universal Testing Machine (mod. 3344, USA). Two slices of each loaf (cut from the central portions of the bread) with diameters of 25 mm were used as test pieces. Test pieces were compressed to 80% for two successive times to obtain two curves, and various properties of the bread crumb including springiness, hardness, chewiness, gumminess and resilience were generated with the Bluehill 2 software (version 2.17). TPA was performed in triplicates per formulation.

4.2.5 Sensory analysis of bread loaves

Consumer sensory analysis was conducted with 36 panellists. The control bread and BGNIF enriched breads were prepared 24 h before sensory evaluation. One slice (5.4 x 5.0 x 1.5 cm) of each bread formulation (control, red, brown, black-eye and brown-eye) was placed individually in an airtight polyethylene bag and randomly coded with a three-digit number. One sample of each formulation was placed on a tray and served to the panellists in a naturally lighted and well-ventilated sensory room. A cup with tap water was provided to reset the palate between tastings. The panellists were requested to give their consent for participation in the study, by signing the provided consent form before evaluating the samples. The panellists were instructed to rate all samples with a five point hedonic rating scale for appearance, crumb colour, crust colour, taste, aroma, texture and overall acceptability of each bread formulation. A score of 1 represented dislike very much and a score of 5 represented like very much. Breads were considered acceptable if their mean values for overall acceptability were 3 (neither like nor dislike) and above.

4.2.6 Statistical analysis

Statistical analysis was performed by testing significant differences between treatments using multivariate analysis of variance (MANOVA). Duncan's multiple range test was used to separate means where differences existed. Kruskal-Wallis test (nonparametric analysis) was used to separate means where differences existed for crumb grain data (IBM SPSS Statistics, version 22, 2013). Principal Component Analysis [PCA] was applied to extract the components that explained the variability in the bread physicochemical data. Sensory data was also subjected to Categorical Principal Component Analysis [CATPCA], to determine if a reduction in variables could be used to differentiate between the control and BGNIF enriched breads (IBM SPSS Statistics, version 22, 2013).

4.3 Results and Discussion

4.3.1 Optimal BGNIF bread

Model fitting

The SLV and textural parameters obtained from the mixture design for determining the optimum loaf formulation is presented in Table 4.2. The summary of the model statistics and regression coefficients is shown in Table 4.3.

The linear mixture model was significant [F(0.23, 0.049) = 8.29; p = 0.0188] in describing the component effects (water, yeast and BGNIF) on SLV. There was only a 1.88% chance that a model F-value this large could occur due to noise. The model lack of fit [F(0.018, 0.049) = 0.36; p = 0.8232] was not significant, indicating a model with adequate goodness-of-fit. This was also confirmed by the adequate precision (6.709) with a value >4 indicating adequate precision and a model which can be used to navigate the design space.

The component effects on gumminess was adequately described by the linear mixture model [F(0.41, 0.068) = 7.15; p = 0.0204). There was only a 2.04% chance that a model F-value this large could occur due to noise. The model had adequate goodness-of-fit as judged by the non-significant model lack of fit [F(0.053, 0.068) = 0.79; p = 0.6406] and adequate precision (6.696), thus this model could be used to navigate the design space.

Resilience and chewiness were also adequately described by the linear mixture model [F(<0.00001, <0.00001) = 6.69; p =0.0530) and [F(32.22, 4.22) = 6.80; p = 0.0229) respectively. The chance that a model F-value this large could occur due to noise was 5.30% for resilience and 2.29% for chewiness. The model goodness-of-fit was adequate for resilience as indicated by the model lack of fit [F(<0.00001, <0.00001) = 1.04; p = 0.6015] and adequate precision (6.501); similarly model lack of fit [F(4.95, 4.22) = 1.17; p = 0.5197] which was not significant and the adequate precision (6.702) for chewiness also showed a model with adequate goodness-of-fit.

All variables were fitted to a linear model, and the residual errors calculated to determine the goodness of model fit. Based on these values, the linear mixture model was found to be suited to determine the effects of the three ingredients (water, yeast and BGNIF) on the specific loaf volume, gumminess, resilience and chewiness of the breads. Normality of data was confirmed with two diagnostic tools – Normality Plot of Residuals and the Box-Cox Plot (not shown). The straight line of the plots which indicates a normal distribution of residuals (Jeirani *et al.*, 2012), showed that a change in transformation would not improve the analysis. The Box-Cox plot which is “the natural log of the sum of the squares of the residuals against lambda” indicates whether lambda should be transformed (Jeirani *et al.*, 2012); and no transformation was recommended for the response variables.

Table 4.2 Specific loaf volume and textural properties for bread optimisation formulations[1]

Formulation	Component variables			Response variables			
	Water %	Yeast %	BGNIF %	Specific loaf volume (ml/g)	Gumminess (N)	Chewiness (N)	Resilience
1	60.0	2.3	10.0	2.83 ± 0.18	1.96 ± 0.43	18.35 ± 3.92	0.036 ± 0.003
2	60.0	2.3	10.0	3.18 ± 0.12	1.70 ± 0.16	15.96 ± 1.39	0.040 ± 0.004
3	60.0	5.3	7.0	3.36 ± 0.06	2.89 ± 0.19	25.88 ± 1.47	0.068 ± 0.008
4	57.0	5.3	10.0	3.54 ± 0.24	2.01 ± 0.24	18.63 ± 2.03	0.051 ± 0.007
5	59.5	4.8	8.0	3.68 ± 0.19	2.36 ± 0.17	22.39 ± 1.93	0.040 ± 0.006
6	57.0	5.3	10.0	3.27 ± 0.23	1.56 ± 0.20	14.66 ± 1.87	0.157 ± 0.210
7	58.5	5.3	8.5	3.66 ± 0.24	2.07 ± 0.12	19.21 ± 1.01	0.057 ± 0.006
8	59.0	4.3	9.0	3.66 ± 0.16	2.17 ± 0.57	20.42 ± 5.10	0.046 ± 0.016
9	58.5	3.8	10.0	3.31 ± 0.14	2.28 ± 0.19	21.78 ± 1.79	0.041 ± 0.010
10	60.0	3.8	8.5	3.33 ± 0.06	2.24 ± 0.65	21.47 ± 6.69	0.043 ± 0.006

[1] BGNIF: Bambara groundnut insoluble dietary fibre

Table 4.3 Regression coefficients and model summary statistics of the linear model for SLV, gumminess, chewiness and resilience of optimisation loaves[1]

Response variables	Regression, R^2	Adjusted regression, R^2	Adequate Precision	Lack of fit p-value
Specific loaf volume (ml/g)	0.734	0.646	6.709	0.823
Gumminess (N)	0.671	0.577	6.696	0.641
Chewiness (N)	0.660	0.563	6.702	0.520
Resilience	0.770	0.655	6.501	0.602

[1] BGNIF: Bambara groundnut insoluble dietary fibre; SLV: specific loaf volume.

Effects of mixture components on specific loaf volume and bread textural parameters

The SLV of formulation 1 was the lowest (2.83 ± 0.18 $ml.g^{-1}$), which highlights the reduction in SLV when the highest amount of BGNIF and the lowest amount of yeast were used. This observation was confirmed by the trace (Piepel) plot (Figure 4.2a) indicating that the SLV was more sensitive to changes in yeast (component B) and BGNIF (component C). The 3D response surface plot (Figure 4.2b) clearly shows that highest SLV was found in the BGNIF vertex (representing lowest amount of fibre) and to some extent in the yeast-BGNIF edge. Lowest SLV was found in the yeast vertex (representing lowest amount of yeast) and the water-yeast edge.

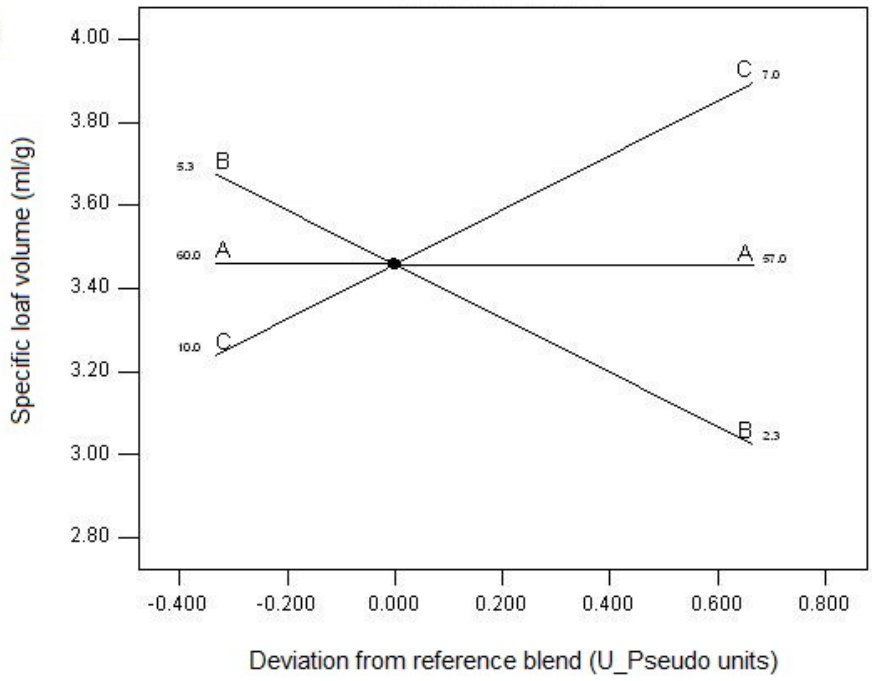
Bread gumminess was the highest in formulation 3 (2.89 ± 0.19 N) where BGNIF was present at the lowest amount (7%), and subsequently decreased when more BGNIF was used. The trace (Piepel) plot and response surface plot for bread gumminess is shown in Figures 4.3a and 4.3b, respectively. These plots show that BGNIF had the greatest effect on gumminess, with an increase in BGNIF leading to lower bread gumminess. The maximum gumminess was located at the BGNIF vertex, which represents the lowest level of BGNIF (7%).

The response surface for bread chewiness was similar to that of bread gumminess. BGNIF (component C) had the greatest effect on chewiness as seen on the trace (Piepel) plot in Figure 4.4a. Increase in BGNIF led to lower bread chewiness. On the response surface (Figure 4.4b) the maximum chewiness was observed at the BGNIF vertex; and as the BGNIF content increased the chewiness decreased towards the water-yeast edge.

The lowest resilience was observed in formulation 1 (0.036 ± 0.003) where water was at the highest level (60%) and yeast at the lowest level (2.3%); in contrast the highest resilience was observed at the lower water level (57%) and highest yeast level (5.3%) as seen in formulation 6 (0.157 ± 0.210). The effects of these two components are clearly shown in the trace (Piepel) plot (Figure 4.5a) where components A (water) and B (yeast) deviated most from the reference blend. This observation was further confirmed by the response surface (Figure 4.5b) where the maximum resilience is seen in the water vertex and the water-BGNIF edge.

Good quality bread is generally characterised by lower gumminess and chewiness, and high specific loaf volume. Higher BGNIF concentrations led to reduced gumminess and chewiness. Specific loaf volume was also slightly reduced with increasing BGNIF concentrations, although still remaining comparable to breads with lower BGNIF content. The incorporation of BGNIF into white bread formulation thus leads to improved quality characteristics, thereby highlighting the great potential of these fibre fractions.

(a)



(b)

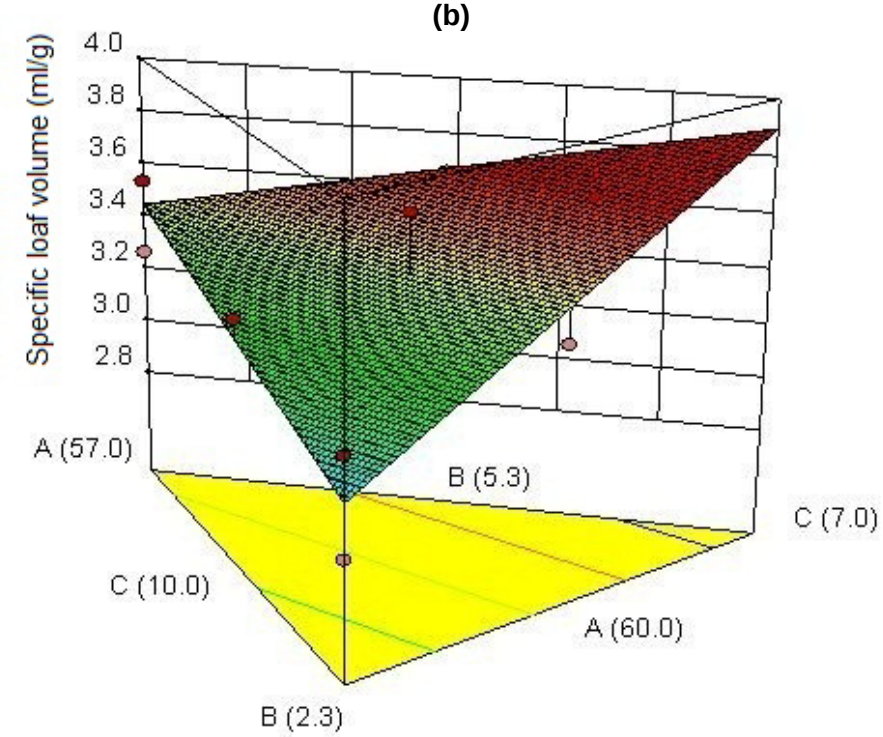
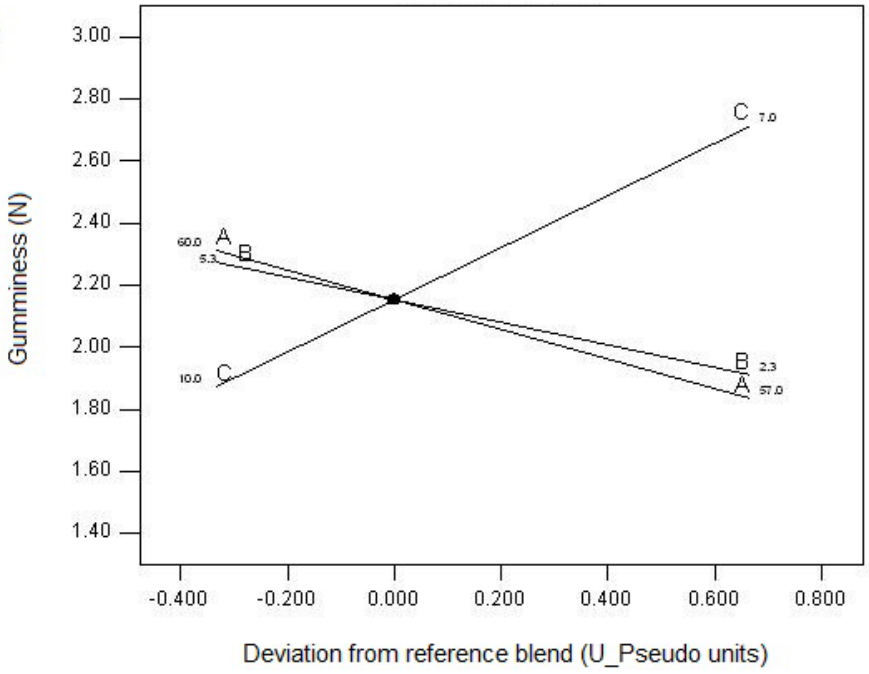


Figure 4.2 **(a)** Trace (Piepel) plot and **(b)** response surface plot for the effect of three components (A: water, B: yeast and C: Bambara groundnut insoluble dietary fibre) on specific loaf volume.

(a)



(b)

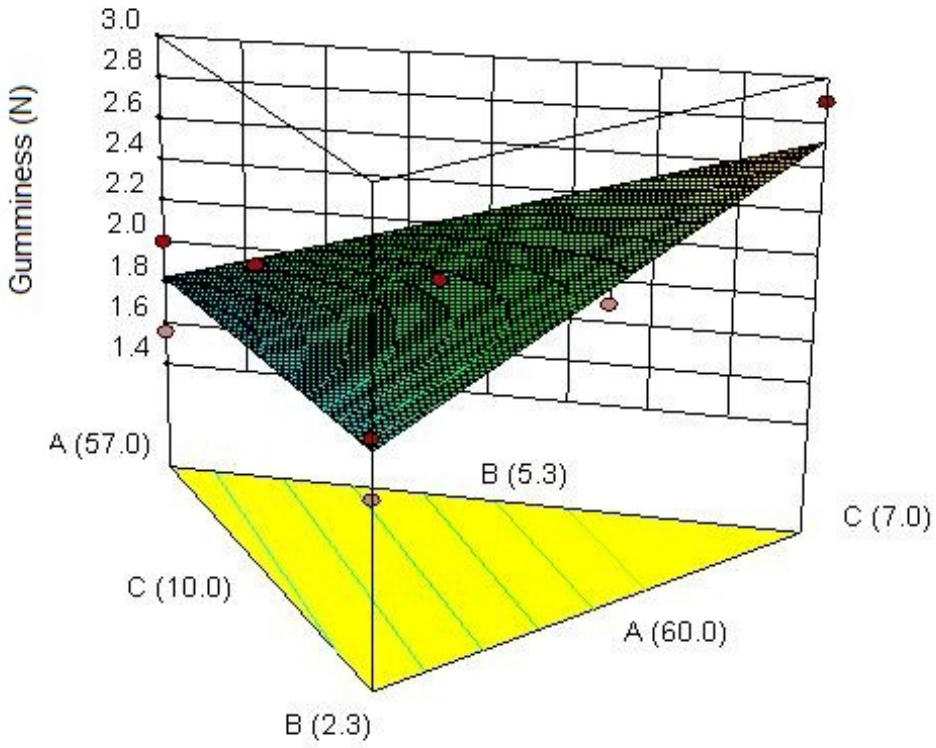
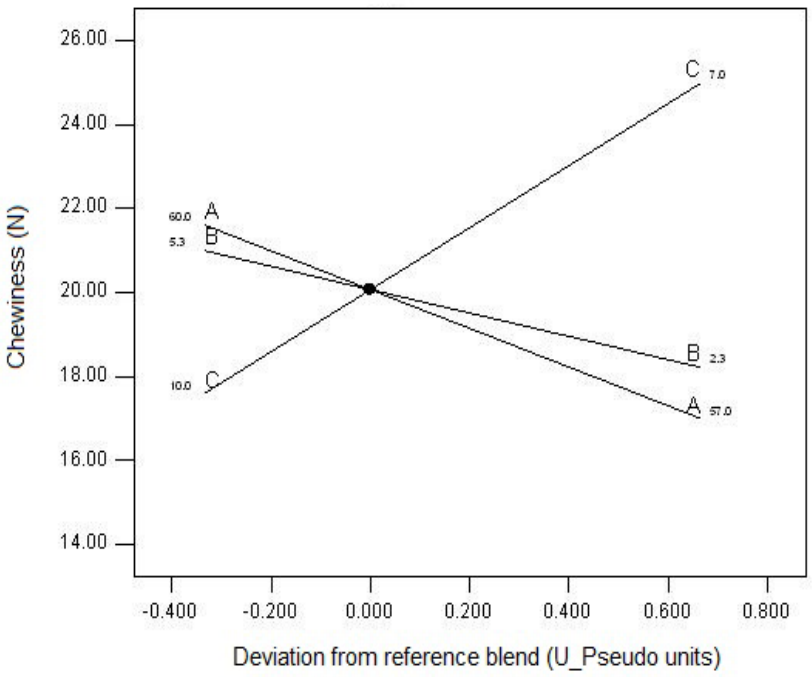


Figure 4.3 **(a)** Trace (Piepel) plot and **(b)** response surface plot for the effect of three components (A: water, B: yeast and C: Bambara groundnut insoluble dietary fibre) on bread gumminess.

(a)



(b)

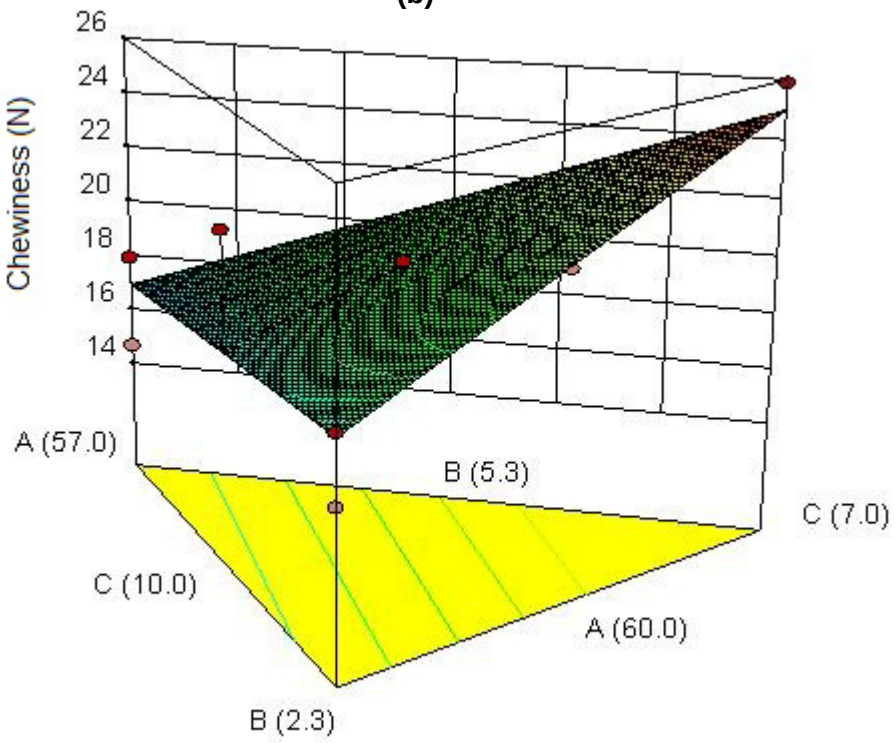
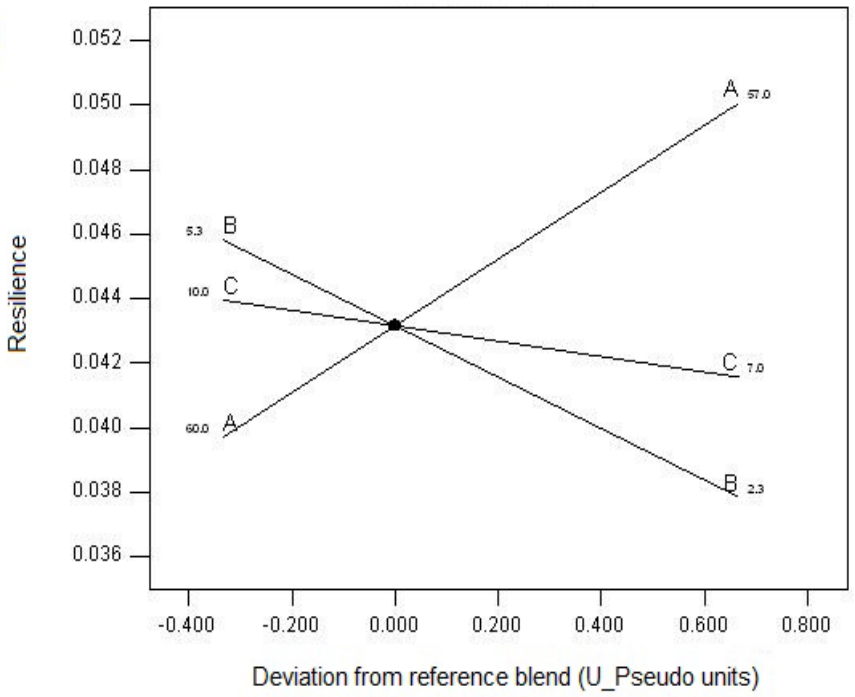


Figure 4.4 **(a)** Trace (Piepel) plot and **(b)** response surface plot for the effect of three components (A: water, B: yeast and C: Bambara groundnut insoluble dietary fibre) on bread chewiness.

(a)



(b)

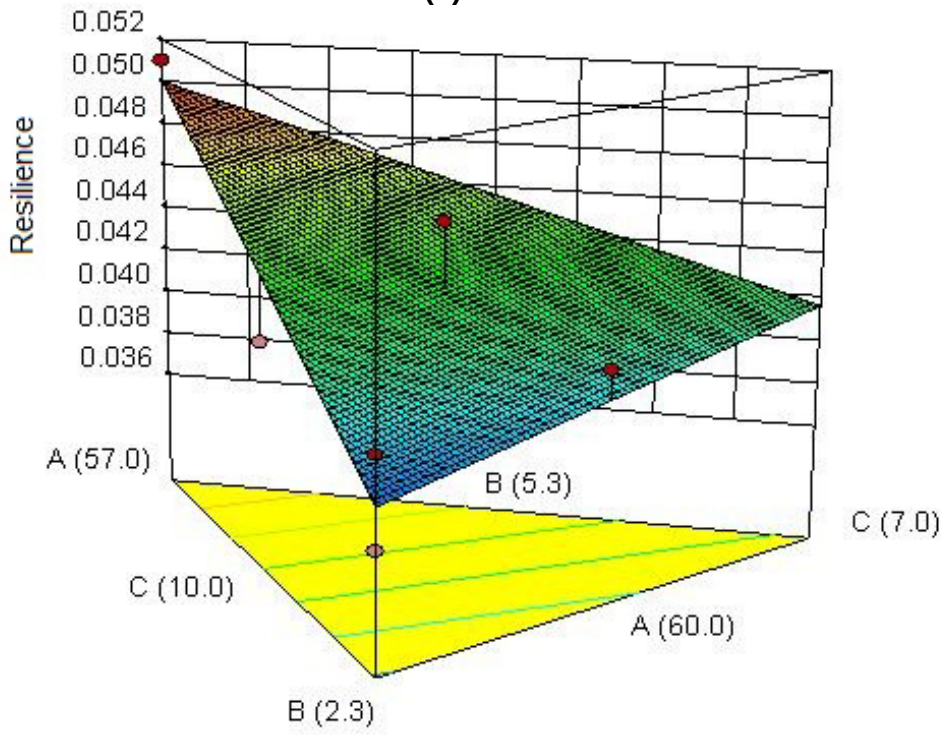


Figure 4.5 **(a)** Trace (Piepel) plot and **(b)** response surface plot for the effect of three components (A: water, B: yeast and C: Bambara groundnut insoluble dietary fibre) on bread resilience.

Optimal bread formulation and model validation

Based on their high regression[1] (R^2), SLV and bread gumminess were taken as the two responses to be jointly optimised by the components. The goal for optimisation of each component was specified as follow: component A (water) in range, component B (yeast) target = 4.3 and component C (BGNIF) target = 8.5, whilst maximising SLV and minimising gumminess. The best optimal solution with a desirability of 0.778 was found as 59.5% for component A (water), 4.3% for component B (yeast) and 8.5% for component C (BGNIF). The predicted responses under these conditions were 3.53 $ml.g^{-1}$ for specific loaf volume and 2.3 N for gumminess. The schematic representation of the optimal formulation and the response surface for desirability of the optimum solution is shown in Figures 4.6 and 4.7, respectively.

The accuracy of the model was evaluated by conducting experiments with the optimum compositions (59.5% water, 4.3% yeast and 8.5% BGNIF). The results obtained were 3.80 ± 0.06 $ml.g^{-1}$ for specific loaf volume and 2.2 ± 0.3 N for gumminess. These results were comparable to the predicted values and thus indicate that the linear model was accurate in determining the optimum formulation for BGNIF enriched bread. The objective to incorporate BGNIF into white bread formulation was then also achieved.

4.3.2 Physicochemical properties of optimal bread with different BGN fibre varieties

Effect of BGNIF on bread crumb grain characteristics

Crumb grain is considered to be important in establishing the quality of bread since the accuracy with which other bread quality attributes are scored is dependent on these characteristics (Scanlon & Zghal, 2001). Visual evaluation of the bread crumbs (Figure 4.8) shows BGNIF enriched breads with denser compact crumbs compared to the control bread. In addition, the threshold images (Figure 4.8) also shows BGNIF enriched breads had more pores which appeared to be smaller compared to the control bread crumb. This observation was also made by Polaki *et al.* (2010) where bread prepared with a percentage of whole oat flour were characterised by the most compact crumb structure and the smallest pores.

The control bread crumb had 319 pores whilst the number of pores in BGNIF breads ranged from 429 – 538. Crumb structure analysis indicated that bread enriched with BGNIF varieties increased the number of pores in the crumb. The pore size distributions shown in Figure 4.9 indicate the percentage of pores in categories. The majority of pores can be grouped into four categories: pores with area < 5.00 mm^2, 5.00 – 24.99 mm^2, 25.00 – 44.99 mm^2 and 45.00 – 64.99 mm^2. The highest amount of pores for all breads was predominantly grouped in the lower area categories. Majority of the pores (75.2%) from the control bread

[1] Regression for resilience was the highest, however specific loaf volume and gumminess are considered more important parameters influencing consumer acceptance of bread.

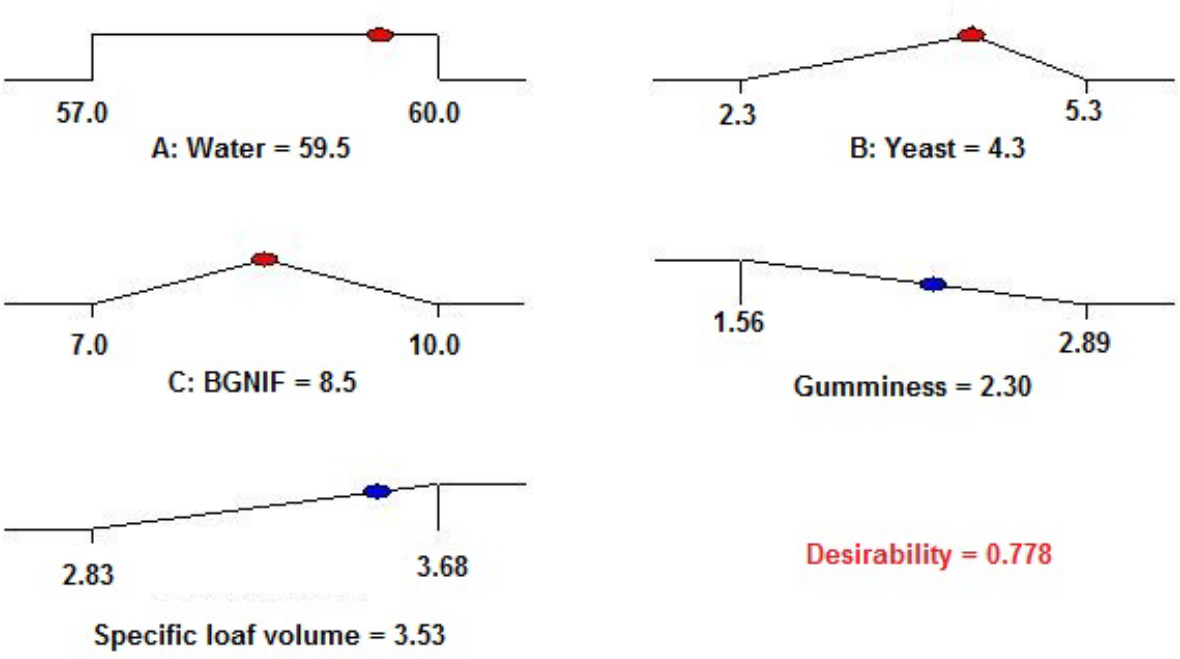


Figure 4.6 Schematic representation of the optimum levels for components (water, yeast and Bambara groundnut insoluble dietary fibre [BGNIF]) and response variables (specific loaf volume and gumminess), and their corresponding desirability.

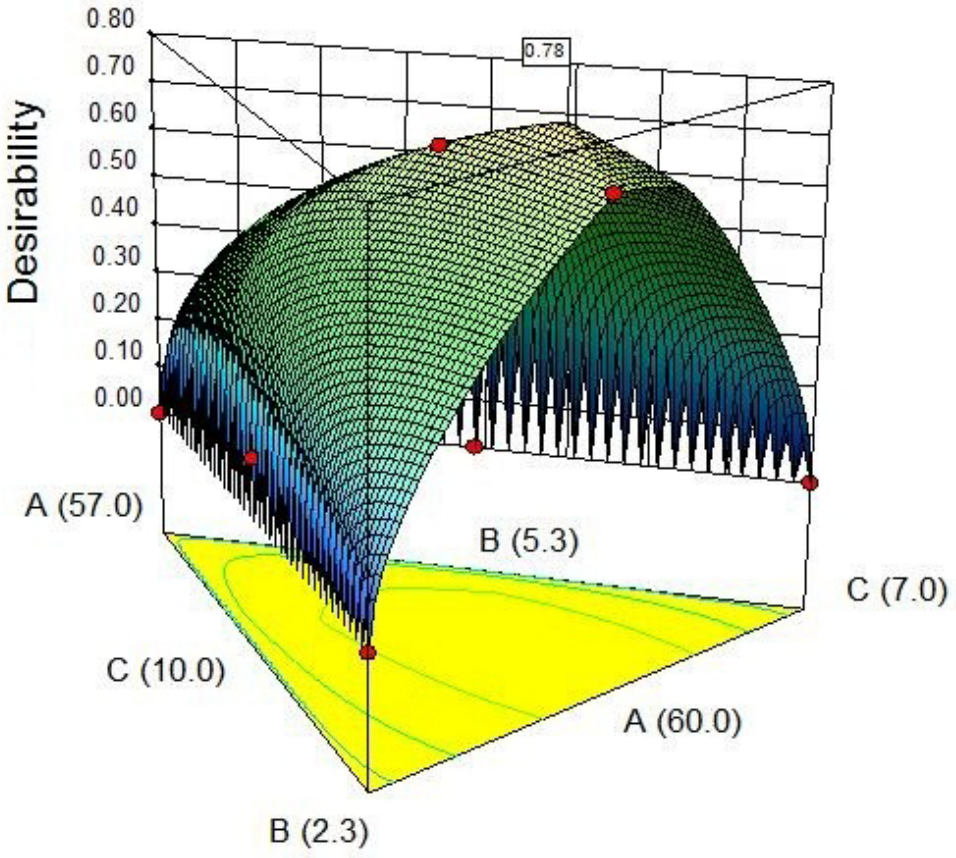


Figure 4.7 Response surface showing desirability at optimum water (A = 59.5%), yeast (B = 4.3%), and Bambara groundnut insoluble dietary fibre (C = 8.5%) levels.

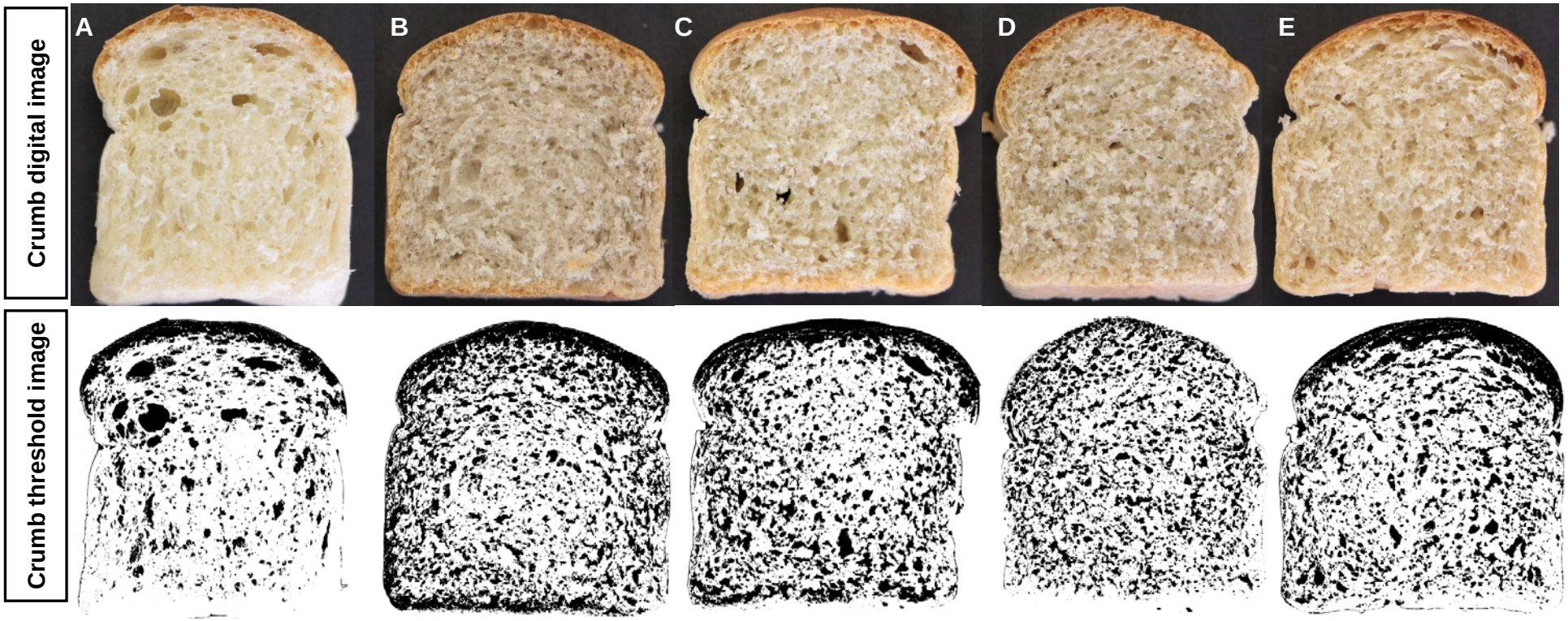


Figure 4.8 Digital and corresponding threshold images of control **(A)** white bread crumb and Bambara groundnut insoluble dietary fibre (**B:** black-eye, **C:** red, **D:** brown, **E:** brown-eye) enriched bread crumbs.

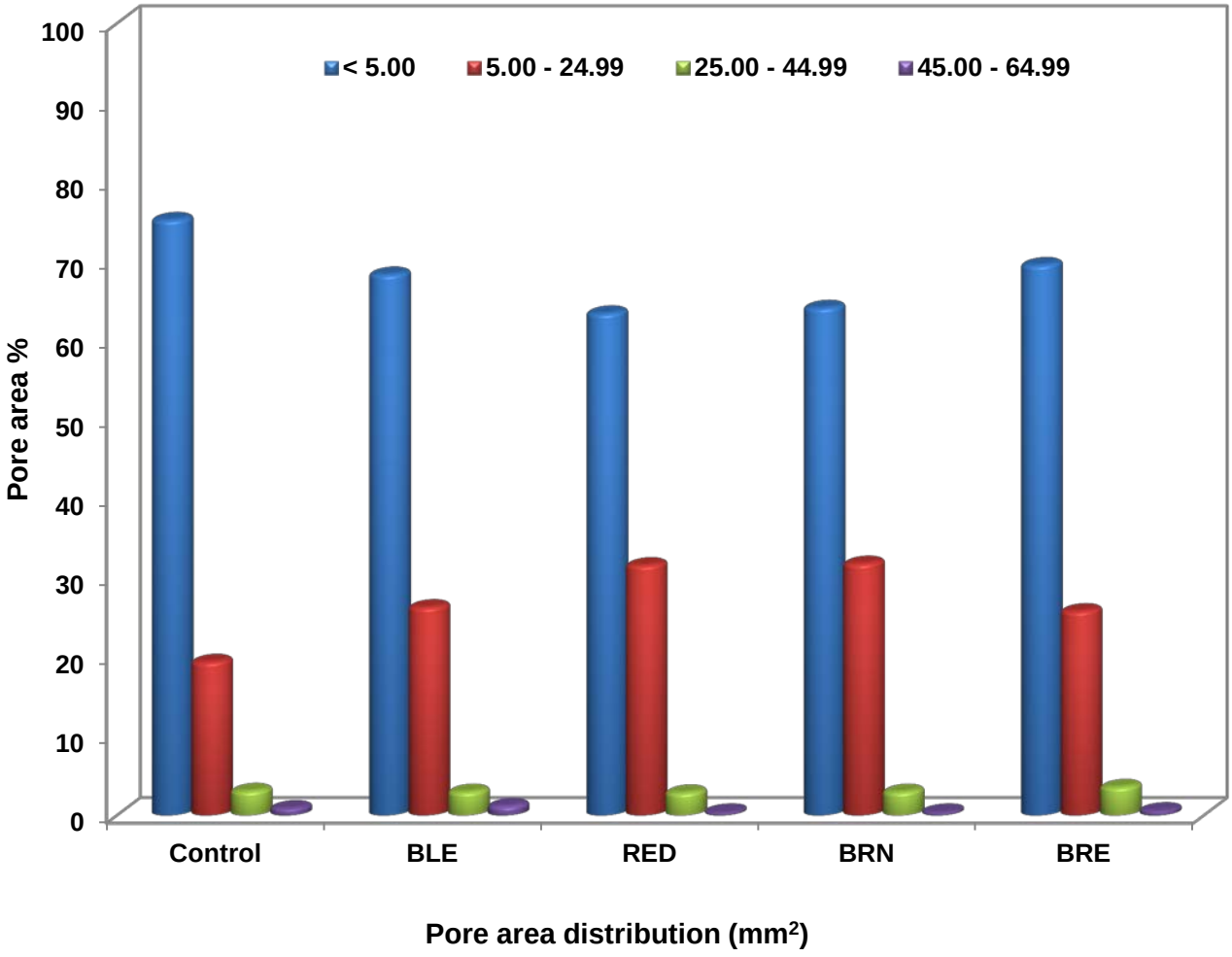


Figure 4.9 Pore area distribution of control and Bambara groundnut insoluble dietary fibre (BLE: black-eye, RED: red, BRN: brown and BRE: brown-eye) enriched bread crumbs.

had areas < 5.00 mm^2, whilst 63.4 – 69.5% of pores from BGNIF enriched breads were categorised in this group with no significant [$p > 0.05$] difference found in the number of pores amongst the different bread crumbs. The number of pores with areas 5.00 – 24.99 mm^2 was 19.4% for the control bread which differed significantly [$p \leq 0.05$] from the number of pores of the BGNIF enriched bread ranging from 25.8 – 31.8%. Fewer pores were found in the higher pore areas with a total representation of 3.1% in the 25.00 – 44.99 mm^2 and 0.7% in the 45.00 – 64.99 mm^2 categories. Bread crumbs seem to exhibit similar distributions. Furthermore black-eye and brown-eye BGNIF bread, and red and brown BGNIF bread crumbs showed pore distributions of a similar nature. The median pore size for all bread crumbs was 2.96 mm^2; red and brown BGNIF breads contained a higher percentage of pores with areas higher than the median whilst the control, brown-eye and black-eye breads contained a higher percentage of pores with areas lower or equal to the median.

Some parameters describing the shape characteristics of the pores from the different bread crumbs are presented in Table 4.4. The feret angle was computed as the average of all pores present per bread, and represents the angle (0 – 180°) of the Feret's diameter which is the longest distance between any two points in a selected area (Ferreira & Rasband, 2012). The feret angle of the control (81.99 ± 45.62°) bread pores were significantly [$p \leq 0.05$] lower compared to that of pores found in BGNIF (91.59 – 94.41°) bread crumbs. Circularity is measured on a scale of 0.0 – 1.0; a value of one indicates a perfect circle whereas values approaching zero indicates an increasing elongated shape. Circularity of the pores ranged from 0.25 ± 0.13 for black-eye bread to 0.28 ± 0.15 for red BGNIF bread, with the only significant [$p \leq 0.05$] difference found between these two varieties. The pores in the crumbs of the control and BGNIF breads were thus not circular in shape. This is further confirmed by the roundness of the pores which measures how far the shape of the pores differs from a circle; pores which are round and thus more uniform in shape have values equal to 1.0 (Polaki *et al.*, 2010). Pores in the control bread had a roundness of 0.52 ± 0.20 with that of BGNIF bread being slightly higher ranging from 0.54 ± 0.18 for brown-eye bread to 0.56 ± 0.18 for red and brown BGNIF bread, respectively. Roundness of pores in the control bread was significantly [$p \leq 0.05$] lower compared to pores in black-eye, red and brown BGNIF breads. This could be attributed to the effect of the fibres on the gluten structure; as suggested by Ishida *et al.* (2002) dietary fibres play a role in disrupting the continuity of the gluten matrix and rounder pores indicates a greater destruction of this matrix. The aspect ratio which indicates the relationship between the pore width and height (Demirkesen *et al.*, 2014) were significantly [$p \leq 0.05$] higher for pores in the control (2.50 ± 2.26) bread crumb. Comparably the aspect ratio did not differ significantly [$p > 0.05$] between the BGNIF enriched breads, ranging from 2.05 ± 1.00 for brown BGNIF bread to 2.20 ± 1.35 for brown-eye BGNIF bread.

Table 4.4 Selected shape descriptors of pores from control and BGNIF enriched bread crumbs[1, 2]

Bread	Feret Angle	Circularity	Roundness	Aspect Ratio
Control	81.99 ± 45.62^{a}	0.27 ± 0.14^{ab}	0.52 ± 0.20^{a}	2.50 ± 2.26^{a}
BLE	94.41 ± 50.87^{b}	0.25 ± 0.13^{a}	0.55 ± 0.17^{b}	2.09 ± 1.13^{b}
RED	92.78 ± 46.06^{b}	0.28 ± 0.15^{b}	0.56 ± 0.18^{b}	2.07 ± 1.17^{b}
BRN	92.45 ± 48.94^{b}	0.26 ± 0.14^{ab}	0.56 ± 0.18^{b}	2.05 ± 1.00^{b}
BRE	91.59 ± 49.23^{b}	0.27 ± 0.15^{ab}	0.54 ± 0.18^{ab}	2.20 ± 1.35^{b}

[1] Values are Mean ± Standard deviation. Means within a column followed by the same letter are not significantly [$p > 0.05$] different.
[2] BGNIF: Bambara groundnut insoluble dietary fibre; BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye.

Effect of BGNIF on moisture, total dietary fibre and polyphenolic composition of wheat bread

The chemical composition of the control and BGNIF enriched breads are shown in Table 4.5. TDF ranged from 7.1 ± 0.2% for brown BGNIF bread to 8.3% for black-eye BGNIF bread, with black-eye and brown-eye (7.5 ± 0.2%) loaves having the highest significant [$p \leq 0.05$] TDF. TDF of BGNIF enriched loaves were significantly [$p \leq 0.05$] higher compared to TDF content of the control white bread (5.0 ± 0.9%). Compared to the amount of BGNIF (8.5%) used in the formulations, the TDF content in the loaves were slightly lower. These results are comparable to studies by Abdul-Hamid & Luan (2000) and Wang *et al.* (2002), where the addition of fibres (defatted rice bran and sugar beet fibre; and carob, pea and inulin fibres, respectively) to white bread resulted in a lower TDF content than expected. This was attributed to the high baking temperature or hydrolysis caused by enzymatic reactions from the yeast (Abdul-Hamid & Luan, 2000). Nonetheless, all BGNIF varieties considerably increased the amount of dietary fibre in the bread when compared to the control loaf. This highlights the potential of BGNIFs as fibre-enriching food ingredients; thereby achieving one of the objectives as specified in this study.

Moisture content of the control loaf (32.2 ± 0.8%) did not differ significantly [$p > 0.05$] from the BGNIF enriched bread loaves. However, moisture content of brown (33.4 ± 0.9%) BGNIF bread differed significantly [$p \leq 0.05$] from red BGNIF bread (30.89 ± 1.43%) with no significant [$p > 0.05$] difference to the other varieties for both. Wang *et al.* (2002) reported higher moisture contents in pea and carob fibre enriched breads, however in their study the significant differences was not statistically analysed. Dalgetty & Baik (2006) also found bread loaves enriched with hull and cotyledon fibres from different legume sources (pea, lentil and chickpea, respectively) had a higher moisture content compared to the control. At the highest substitution rate (7%) for hull and insoluble cotyledon fibres, the bread moisture content ranged from 45.3 – 48.9% compared to the lower 44.5% for the control bread. The contrasting trend obtained in this study could be attributed to the different fibre types used (insoluble dietary fibre from the whole seed as opposed to dietary fibre from specific fractions of the seed) and their specific hydration properties; which possibly shows to a higher water absorption capacity of the BGNIFs thereby creating a lower moisture content an invariably improving the shelf life of the bread.

Trace amounts of condensed tannins [CT] were present in all loaves. No significant [$p > 0.05$] difference was observed for CT content in brown-eye (0.0004 ± 0.0001 $mg.g^{-1}$) BGNIF bread and the control bread (0.0003 ± 0.0001 $mg.g^{-1}$). Condensed tannins differed significantly [$p \leq 0.05$] between all BGNIF enriched breads with the highest amount found in red (0.0023 ± 0.0002 $mg.g^{-1}$) BGNIF bread. The polyphenol [PP] content in brown BGNIF bread (5.06 ± 0.40 $mg.g^{-1}$ gallic acid equivalents [GAE]) differed significantly [$p \leq 0.05$] from the control bread (4.26 ± 0.27 $mg.g^{-1}$ GAE), whilst the bread loaves enriched with the other

Table 4.5 Some chemical properties of control and BGNIF enriched breads[1, 2]

Bread	Moisture (% d.m.)	TDF (% d.m.)	Condensed tannins (mg.g^{-1})	Polyphenols (mg.g^{-1} GAE)
Control	32.2 ± 0.8^{ab}	5.0 ± 0.9^{a}	0.0003 ± 0.0001^{a}	4.26 ± 0.27^{a}
BLE	32.6 ± 1.9^{ab}	8.3 ± 0.0^{b}	0.0006 ± 0.0001^{b}	4.73 ± 0.13^{ab}
RED	30.9 ± 1.4^{a}	7.2 ± 0.8^{c}	0.0023 ± 0.0002^{c}	4.37 ± 0.19^{a}
BRN	33.4 ± 0.9^{b}	7.1 ± 0.2^{c}	0.0016 ± 0.0001^{d}	5.06 ± 0.40^{b}
BRE	33.0 ± 0.8^{ab}	7.5 ± 0.2^{bc}	0.0004 ± 0.0001^{a}	4.34 ± 0.18^{a}

[1] Values are Mean ± Standard deviation. Means within a column followed by the same letter are not significantly [$p > 0.05$] different.
[2] BGNIF: Bambara groundnut insoluble dietary fibre; BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye; TDF: total dietary fibre; GAE: gallic acid equivalents; d.m. dry matter basis

varieties did not differ significantly [$p > 0.05$] from the control. PP in the brown BGNIF bread was also significantly [$p \leq 0.05$] higher compared to the amount found in red (4.37 ± 0.19 $mg.g^{-1}$ GAE) and brown-eye (4.34 ± 0.18 $mg.g^{-1}$ GAE) BGNIF loaves. Both PP and CT content were greatly reduced in the BGNIF breads when compared to the amounts present in the fibres only (see section 3.3.2, page 58 and Jideani & Diedericks, 2013). The negligible quantities of CT in the BGNIF breads could be positively highlighted as they are regarded as anti-nutritional compounds (Saura-Calixto & Bravo, 2001). The effect of baking on phenolic compounds have been investigated by several researchers (Asami *et al.*, 2003; Wang & Zhou, 2004; Sudha *et al.*, 2007; Rupasinghe *et al.*, 2008; Ragaee *et al.*, 2011; Blandino *et al.*, 2013). The overall consensus is that the increase or decrease in PP content after baking is dependent on the individual compounds and their resistance to thermal degradation. Wang & Zhou (2004) also suggested that a reduced phenolic content could be attributed to interactions of wheat proteins with phenolic antioxidants through hydrogen bonding during dough preparation.

Effect of BGNIF on specific loaf volume and texture parameters of wheat bread

Specific loaf volume ranged from 3.33 ± 0.04 $ml.g^{-1}$ to 3.85 ± 0.02 $ml.g^{-1}$ for red and brown-eye BGNIF enriched breads, respectively. As shown in Figure 4.10, all BGNIF enriched loaves had significantly [$p \leq 0.05$] lower specific loaf volumes compared to the control bread (4.16 ± 0.05 $ml.g^{-1}$). Red and black-eye enriched bread yielded the lowest specific loaf volumes and brown and brown-eye [$p > 0.05$] breads yielded the highest specific loaf volumes. The reduction in specific loaf volumes of breads enriched with BGNIF are in agreement with results found in other studies where dietary fibre addition resulted in lower specific loaf volumes. Dalgetty & Baik (2006) observed that bread enriched with pea, lentil and chickpea fibre fractions resulted in lower specific loaf volumes compared to the control bread, and more so when increased amounts were used. This same observation was reported by Wang *et al.* (2002) and Gómez *et al.* (2003) where dietary fibres from various origins (carob, inulin, cellulose, orange, wheat, etc.) were used. This effect of dietary fibre on bread specific volume is attributed to the fibre gluten interaction which results in a diluted gluten protein, and the consequent interference in the formation of an optimal gluten matrix during fermentation and baking (Blandino *et al.*, 2013).

Depending on the source and type of fibre fraction used, the effect of dietary fibre addition could have a positive or negative influence on the textural properties of breads. Five texture parameters (hardness/firmness, chewiness, resilience, gumminess and springiness) were measured to determine the textural characteristics of the respective bread loaves and results are reported in Table 4.6. Bread without any added fibre had a significantly [$p \leq 0.05$] firmer crumb (9.69 ± 0.19 N) compared to fibre-enriched breads. Brown BGNIF (4.13 ± 0.74 N) bread had the significantly [$p \leq 0.05$] softest crumb with no significant [$p > 0.05$] difference

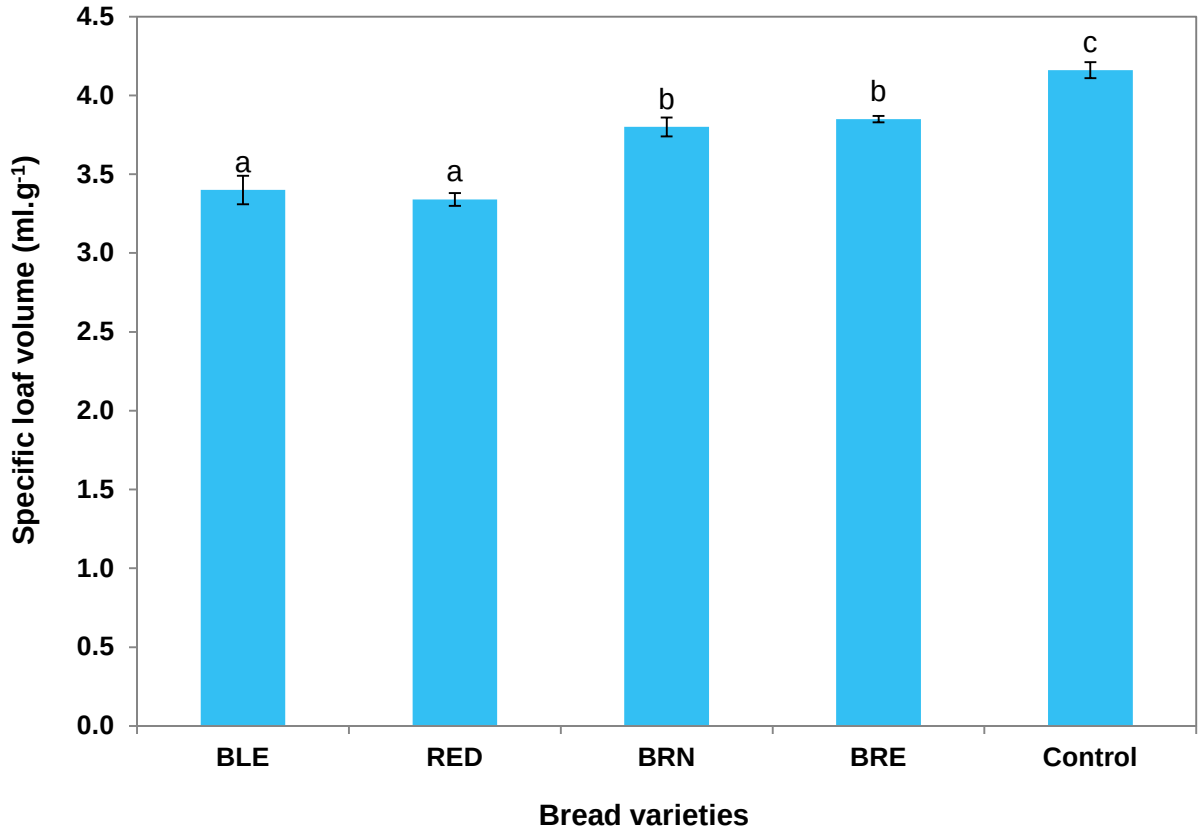


Figure 4.10 Specific loaf volume of control and Bambara groundnut insoluble dietary fibre enriched bread (BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye). Values are Mean ± Standard deviation. Columns with the same letter are not significantly different [p > 0.05].

in crumb firmness between the other BGNIF enriched breads. Crumb firmness is considered an important parameter used in the evaluation of bread staling development (Mondal & Datta, 2008). As reported in literature, the effect of dietary fibre addition on crumb firmness varies amongst different fibre sources. Defatted rice bran fibre and a commercial rice bran fibre resulted in firmer bread crumbs which increased in firmness at increasing fibre levels (Abdul-Hamid & Luan, 2000). Wang *et al.* (2002) reported increased hardness for inulin enriched bread, whereas carob and pea fibres resulted in softer bread crumbs compared to the control bread. Gómez *et al.* (2003) reported crumb firmness of pea fibre enriched bread to be similar to the control bread at 0 and 24 h after baking. Interestingly a type of wheat fibre resulted in the firmest bread crumb when measured directly after baking, but after 72 h this bread crumb was the softest. Pea, lentil and chickpea hull fibres included in bread at different levels (3 – 7%) resulted in the firmness of bread crumb at day 1 of baking being in a similar range (1.0 – 1.7 N) to the control bread (1.3 N) (Dalgetty & Baik, 2006). Sabanis *et al.* (2009) noted that fibre-enriched breads with lower crumb firmness could be attributed to a delay in starch retrogradation which results from a possible hydrogen bonding between starch and fibre. These results show that dietary fibres from legume sources could lead to softer bread crumbs and are of consequent importance when considering the means of increasing the shelf life of breads. It is thus shown that the addition of BGNIFs to white bread could translate to a longer shelf life of this product.

BGNIF enriched bread ranged from 20.58 ± 3.45 N to 38.64 ± 4.82 N for chewiness and differed significantly [$p \leq 0.05$] from the control (79.53 ± 1.89 N) bread crumb. For chewiness, gumminess and springiness, a trend was observed where no significant [$p > 0.05$] difference were found amongst black-eye and brown BGNIF loaves and red and brown-eye loaves, respectively. Chewiness is defined as “the energy required for chewing a solid food until it is ready for swallowing” (Sahin & Sumnu, 2006b). The lower chewiness of BGNIF enriched bread crumbs could thus be positively highlighted, as less energy would be required to chew these breads as compared to the bread without any added BGNIF. Similarly, the control bread had significantly [$p \leq 0.05$] highest gumminess (8.47 ± 0.21 N) and springiness (8.29 ± 0.45 mm), whilst these parameters were significantly [$p \leq 0.05$] lowest for black-eye (2.63 ± 0.32 N and 6.41 ± 0.42 mm, respectively) and brown (2.16 ± 0.31 N and 6.40 ± 0.13 mm, respectively) BGNIF enriched breads. No significant [$p > 0.05$] difference was found between resilience of the control (0.071 ± 0.005) and black-eye (0.063 ± 0.008) bread, and lowest significant [$p \leq 0.05$] resilience was observed in crumbs of brown (0.033 ± 0.009) and brown-eye (0.038 ± 0.006) BGNIF bread. Springiness and resilience are elasticity parameters which refer to the physical spring back of a sample after deformation and how well a sample returns to its original position, respectively (Texture Technologies, 2013). These parameters were slightly lower in some BGNIF enriched breads when compared to the control bread. Wang *et al.* (2002) also reported lower chewiness and

Table 4.6 Texture parameters of control and BGNIF enriched bread loaves[1, 2]

Bread	Hardness/ Firmness (N)	Chewiness (N)	Resilience	Gumminess (N)	Springiness (mm)
Control	9.69 ± 0.19[a]	79.53 ± 1.89[a]	0.071 ± 0.005[a]	8.47 ± 0.21[a]	8.29 ± 0.45[a]
BLE	6.07 ± 0.39[b]	24.64 ± 3.13[b]	0.063 ± 0.008[ab]	2.63 ± 0.32[b]	6.41 ± 0.42[b]
RED	6.75 ± 0.84[b]	37.10 ± 6.78[c]	0.055 ± 0.008[b]	3.89 ± 0.69[c]	7.55 ± 0.30[c]
BRN	4.13 ± 0.74[c]	20.58 ± 3.45[b]	0.033 ± 0.009[c]	2.16 ± 0.31[b]	6.40 ± 0.13[b]
BRE	5.92 ± 0.31[b]	38.64 ± 4.82[c]	0.038 ± 0.006[c]	4.04 ± 0.50[c]	7.55 ± 0.29[c]

[1] Values are Mean ± Standard deviation. Means within a column followed by the same letter are not significantly [p > 0.05] different.
[2] BGNIF: Bambara groundnut insoluble dietary fibre; BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye.

resilience for carob and pea fibre enriched breads, whilst an increase in springiness was observed. However, the authors did not note statistical differences thereby making it difficult to compare to results found in this study.

Effect of BGNIF on wheat bread colour parameters

Colour is an important parameter which contributes to the consumer acceptability of baked products; and depending on the type and amount of fibre added relative colour changes can occur (Cappa *et al.*, 2013). The effect of BGNIF on bread crumb colour is summarised in Table 4.7. Crumb colour parameters of BGNIF enriched breads ranged from 77.72 – 82.03 for lightness, 2.00 – 2.46 for redness, 7.32 – 9.66 for yellowness, 7.72 – 9.89 for chroma and 71.43 – 77.74° for hue angle. The lightness (L* = 85.24 ± 0.25), redness (a* = 1.22 ± 0.05) and hue angle (h = 82.95 ± 0.23°) for the control bread crumb differed significantly [$p \leq 0.05$] from all BGNIF breads. Compared to the other varieties, brown-eye BGNIF bread crumb (L* = 82.03 ± 0.11) was significantly [$p \leq 0.05$] lighter with a lightness (L*) close to that of the control bread crumb. Interestingly, black-eye BGNIF which was characterised with the significantly [$p \leq 0.05$] highest L* (see section 3.3.2, page 57), resulted in a slightly darker bread crumb (L* = 79.90 ± 0.80). The influence of the pigment components in BGN hulls on end product colour is also clearly seen in Jideani & Murevanhema (2013), where BGN milk produced from BGN flour had higher or lower lightness corresponding to the seed colour; black-eye and brown-eye BGN milk consequently had higher lightness. Highest significant [$p \leq 0.05$] redness was observed in red BGNIF bread crumb (a* = 2.46 ± 0.02) with significantly [$p \leq 0.05$] lower redness for black-eye (a* = 2.10 ± 0.12) and brown (a* = 2.00 ± 0.07) bread crumbs. The control (b* = 9.83 ± 0.21) and black-eye (b* = 9.66 ± 0.32) BGNIF bread crumbs had significantly [$p \leq 0.05$] highest yellowness, whilst yellowness of brown-eye (b* = 9.38 ± 0.13) BGNIF crumb were similar [$p > 0.05$] to black-eye bread crumb. The higher b* values (when compared to the lower a* values) also shows that the yellow pigment is more prominent in the BGNIF varieties. This is also confirmed as the hue angle indicated all bread crumbs to be closest to the yellow chromaticity coordinate (+b* = 90°). Hue angle was significantly [$p \leq 0.05$] highest for the control (h = 82.95 ± 0.23°) bread crumb, and significantly [$p \leq 0.05$] lowest for red (h = 71.43 ± 0.07°) BGNIF bread crumb. No significant [$p > 0.05$] difference was observed for chroma of the control (C* = 9.91 ± 0.20), black-eye (C* = 9.89 ± 0.33) and brown-eye BGNIF (C* = 9.66 ± 0.13) bread crumbs; whilst red BGNIF (C* = 7.72 ± 0.09) bread crumb had significantly [$p \leq 0.05$] lowest chroma indicating a slightly duller crumb colour. The crumb which is not exposed to as high temperatures as the crust generally reflects the colour of the fibre added to the bread (Gómez *et al.*, 2003). This is clearly seen with the red BGNIF variety, as all colour parameters of this bread crumb differed most from the control bread. Dalgetty & Baik (2006) also similarly found that lentil hulls with a naturally dark appearance resulted in a darker bread crumb.

Table 4.7 Effect of BGNIF on bread crumb colour parameters[1, 2]

Bread	L*	a*	b*	C*	h (angle)
Control	85.24 ± 0.25^{a}	1.22 ± 0.05^{a}	9.83 ± 0.21^{a}	9.91 ± 0.20^{a}	82.95 ± 0.23^{a}
BLE	79.90 ± 0.80^{b}	2.10 ± 0.12^{b}	9.66 ± 0.32^{ab}	9.89 ± 0.33^{a}	77.74 ± 0.60^{b}
RED	77.72 ± 0.32^{c}	2.46 ± 0.02^{c}	7.32 ± 0.09^{c}	7.72 ± 0.09^{b}	71.43 ± 0.07^{c}
BRN	80.71 ± 0.49^{b}	2.00 ± 0.07^{b}	7.94 ± 0.04^{d}	8.19 ± 0.05^{c}	75.90 ± 0.42^{d}
BRE	82.03 ± 0.11^{d}	2.31 ± 0.02^{d}	9.38 ± 0.13^{b}	9.66 ± 0.13^{a}	76.20 ± 0.13^{d}

[1] Values are Mean ± Standard deviation. Means within a column followed by the same letter are not significantly [p > 0.05] different.
[2] BGNIF: Bambara groundnut insoluble dietary fibre; BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye; L*: lightness; a*: red (+a*) to green (-a*) range; b*: yellow (+b*) to blue (-b*) range; C*: chroma; h: hue angle.

Crust colour parameters of the control and BGNIF breads are shown in Table 4.8. Lightness of the control (L* = 73.50 ± 0.34) bread crust was significantly [$p \leq 0.05$] highest, whilst crust of BGNIF enriched breads were slightly darker ranging from 69.91 – 71.85 with a significant [$p \leq 0.05$] difference between red (L* = 69.91 ± 1.34) and brown (L* = 71.85 ± 0.21) BGNIF bread crusts. Lowest significant [$p \leq 0.05$] redness was observed in the crust of control (a* = 5.27 ± 0.11) and brown-eye (a* = 5.35 ± 0.11) breads with the highest [$p \leq 0.05$] redness observed for black-eye (a* = 6.73 ± 0.28) bread crust. The crust of black-eye BGNIF bread also exhibited highest significant [$p \leq 0.05$] yellowness (b* = 9.55 ± 0.50), whereas yellowness of the control (b* = 7.11 ± 0.23) bread and brown (b* = 7.13 ± 0.19) and brown-eye (b* = 6.38 ± 0.48) BGNIF bread crusts were similar [$p > 0.05$]. Chroma of the black-eye (C* = 11.69 ± 0.31) bread crust were the highest [$p \leq 0.05$] whilst chroma for control (C* = 8.86 ± 0.23) and brown-eye (C* = 8.33 ± 0.49) breads indicated crusts with duller colour [$p > 0.05$]. Hue angle for brown-eye (h = 49.88 ± 1.06°) crust differed significantly [$p \leq 0.05$] from that of the control (h = 53.35 ± 0.79°) bread crust, with no significant [$p > 0.05$] difference to the control bread for the other BGNIF enriched breads. The colour parameters for all breads indicated crusts which are yellowish-red in colour. As noted by Gómez *et al.* (2003), the inherent colour of fibres have little influence on the crust colour of bread, as the colour formation of crusts are typically associated with caramelisation and Maillard reactions. However, significant differences between the crust colour parameters for the control and BGNIF enriched breads shows that BGNIF varieties have some influence on bread crust colour.

Furthermore, the colour difference (ΔE) with reference to the control bread shows the overall influence of BGNIF on the bread colour. As seen in Figure 4.11, crumb ΔE was significantly [$p \leq 0.05$] lowest for the brown-eye (3.43 ± 0.20) BGNIF bread and significantly [$p \leq 0.05$] highest for red (8.03 ± 0.51) BGNIF bread. Crust ΔE was significantly [$p \leq 0.05$] lowest for brown (1.72 ± 0.42) and brown-eye (2.44 ± 0.78) BGNIF breads, and significantly highest for black-eye (3.96 ± 0.53) and red (4.00 ± 1.23) breads. These results are somewhat in agreement with results found by Gómez *et al.* (2003), where lighter/white fibres such as cellulose and pea fibres resulted in bread crumbs with similar colour to the control bread, and the darker coffee and cocoa fibres resulted in bread crumbs with the highest colour differences. Also as noted earlier, crust colour could be attributed to Maillard/caramelisation reactions; this observation is seen where the black-eye (considered a light fibre) crust difference was similar [$p > 0.05$] to the red (darker) BGNIF bread crust difference.

Table 4.8 Effect of BGNIF on bread crust colour parameters[1, 2]

Bread	L*	a*	b*	C*	h (angle)
Control	73.50 ± 0.34^{a}	5.27 ± 0.11^{a}	7.11 ± 0.23^{a}	8.86 ± 0.23^{ab}	53.35 ± 0.79^{ab}
BLE	71.05 ± 1.18^{bc}	6.73 ± 0.28^{b}	9.55 ± 0.50^{b}	11.69 ± 0.31^{c}	55.44 ± 2.09^{b}
RED	69.91 ± 1.34^{c}	6.16 ± 0.19^{c}	8.08 ± 0.68^{c}	10.17 ± 0.53^{d}	52.57 ± 2.58^{abc}
BRN	71.85 ± 0.21^{b}	5.64 ± 0.17^{d}	7.13 ± 0.19^{a}	9.10 ± 0.24^{b}	51.66 ± 0.45^{ac}
BRE	71.19 ± 0.42^{bc}	5.35 ± 0.11^{ad}	6.38 ± 0.48^{a}	8.33 ± 0.49^{a}	49.88 ± 1.06^{c}

[1] Values are Mean ± Standard deviation. Means within a column followed by the same letter are not significantly [$p > 0.05$] different.
[2] BGNIF: Bambara groundnut insoluble dietary fibre; BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye; L*: lightness; a*: red (+a*) to green (-a*) range; b*: yellow (+b*) to blue (-b*) range; C*: chroma; h: hue angle; ΔE: colour difference between control and enriched breads.

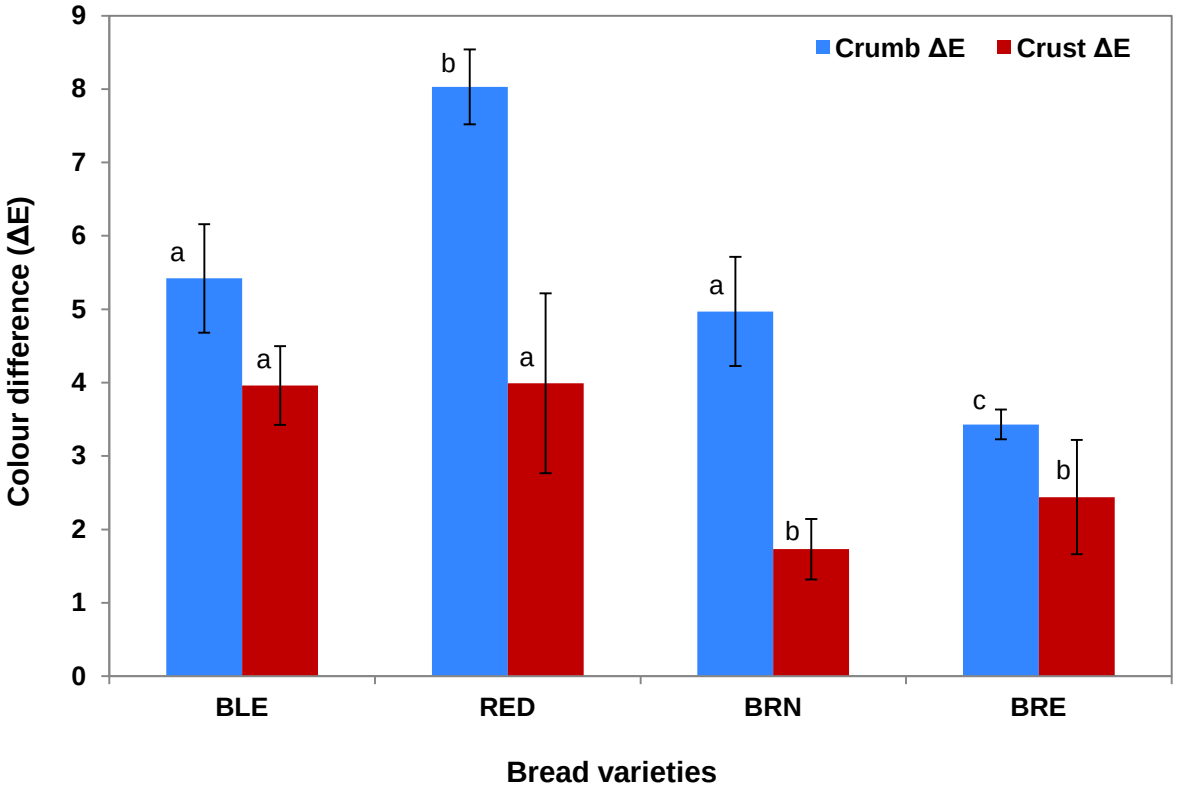


Figure 4.11 Crumb and crust colour difference of Bambara groundnut insoluble dietary fibre enriched bread (BLE: black-eye; RED: red; BRN: brown; BRE: brown-eye) from control bread. Values are Mean ± Standard deviation. Same coloured columns with the same letter are not significantly different [p > 0.05].

4.3.3 Principal components explaining variability in bread physicochemical properties

The suitability of data reduction by PCA was confirmed by the correlation matrix which revealed high correlations between several variables. Furthermore, the Kaiser-Meyer-Olkin [KMO] measure (0.631) was higher than the recommended minimum of 0.6, and the Bartlett's test was significant [$p \leq 0.05$]. The factorability of the correlation matrix was thus supported and PCA was applied.

PCA with Varimax rotation (Table 4.9) showed that variation in bread physicochemical properties of the control and BGNIF enriched breads could be explained by three components with eigenvalues greater than one. Component 1 represented 36.5% of the variability, and was mainly determined by the bread textural properties (hardness/firmness, springiness, chewiness, gumminess and resilience). Component 2 represented 30.5% of variation, with specific loaf volume and bread (crumb and crust) lightness loading strongly on this component; and 25.8% of variation was explained by component 3 with crumb colour parameters (chroma, yellowness and hue) being the main determinants. All three components accounted for a cumulative variation of 92.8%.

The score plot for the first two components (Figure 4.12) shows that the control loaves and red BGNIF loaves were clearly separated by component 1, thus indicating that the differences between these two breads might be attributed to differences in their textural properties. The brown, brown-eye and black-eye BGNIF loaves grouped together on or in close proximity of the zero line for component 1, thus indicating that textural properties does not clearly distinguish between these bread varieties. With respect to component 2 the red BGNIF and control loaves were close, whereas the brown BGNIF bread was clearly separated from these two varieties. Specific loaf volume and bread lightness could thus be used to differentiate between these bread varieties. Brown-eye and black-eye BGNIF loaves were in close proximity situated at the zero line of component 2, thus differentiation between these bread varieties based on specific loaf volume and bread lightness would not be adequate. The score plot for components 1 and 3 (Figure 4.13) again shows a clear differentiation between the red and control loaves with respect to textural properties (component 1). Component 3 however does not differentiate between the red and control loaves; whereas the black-eye BGNIF bread are clearly separated from the brown and brown-eye loaves, thus indicating that crumb colour parameters can be used to differentiate between these bread varieties. The score plot (Figure 4.14) for the second and third components shows grouping of the red and control loaves located positively with respect to component 2, and grouping of brown and black-eye loaves located negatively with respect to component 2. The differences between these bread varieties can thus be explained by specific loaf volume and bread lightness. With respect to component 3, as noted before no

Table 4.9 Coefficient correlations between variables and components for physicochemical properties of control and BGNIF enriched loaves[1]

	Component		
Variable	**1**	**2**	**3**
Hardness/Firmness	0.937	0.143	0.302
Springiness	0.870	0.286	-0.122
Chewiness	0.868	0.450	0.175
Gumminess	0.865	0.449	0.188
Resilience	0.743	-0.247	0.509
Specific loaf volume	0.238	0.943	0.143
Crumb lightness	0.349	0.821	0.416
Crust lightness	0.143	0.792	0.355
Crumb chroma	0.125	0.295	0.908
Crumb yellowness	0.139	0.342	0.903
Crumb hue	0.310	0.628	0.667

[1] BGNIF: Bambara groundnut insoluble dietary fibre.

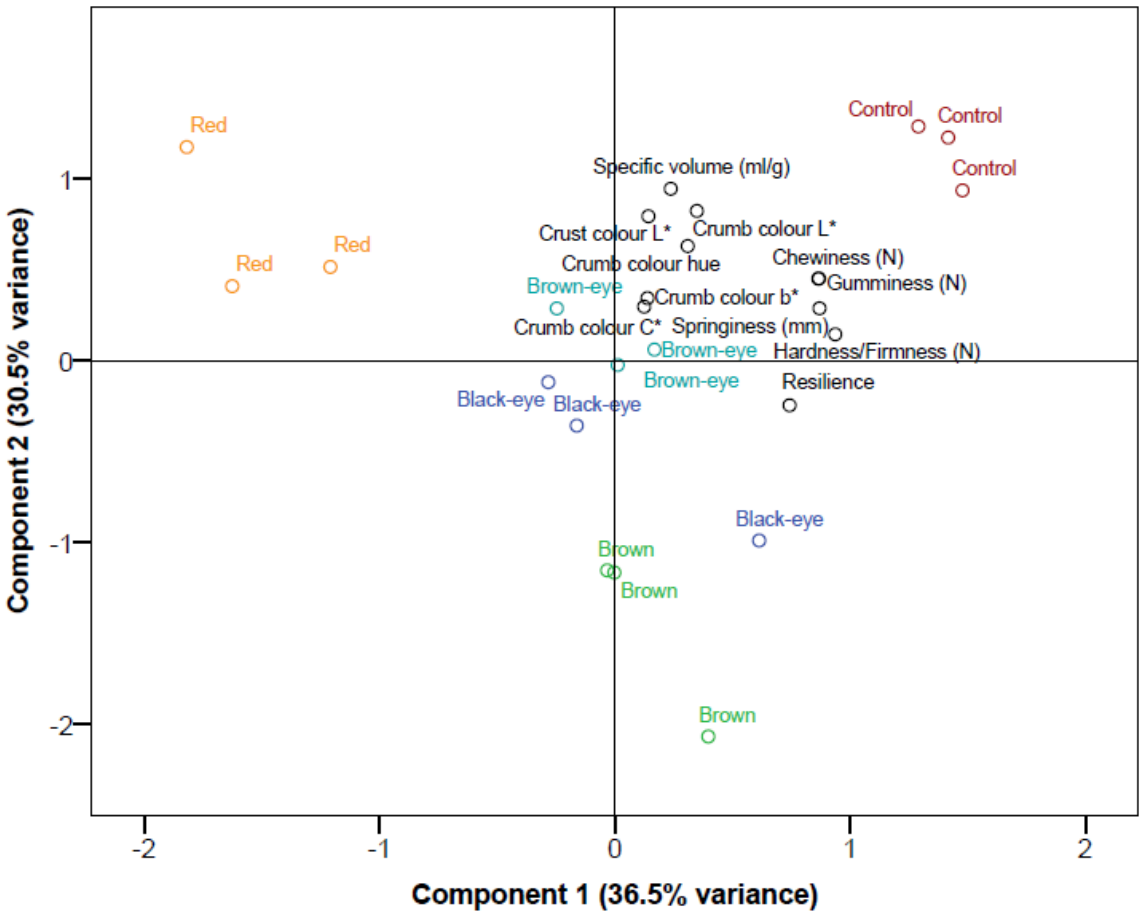


Figure 4.12 Score plot showing differentiation of bread varieties with respect to components 1 and 2.

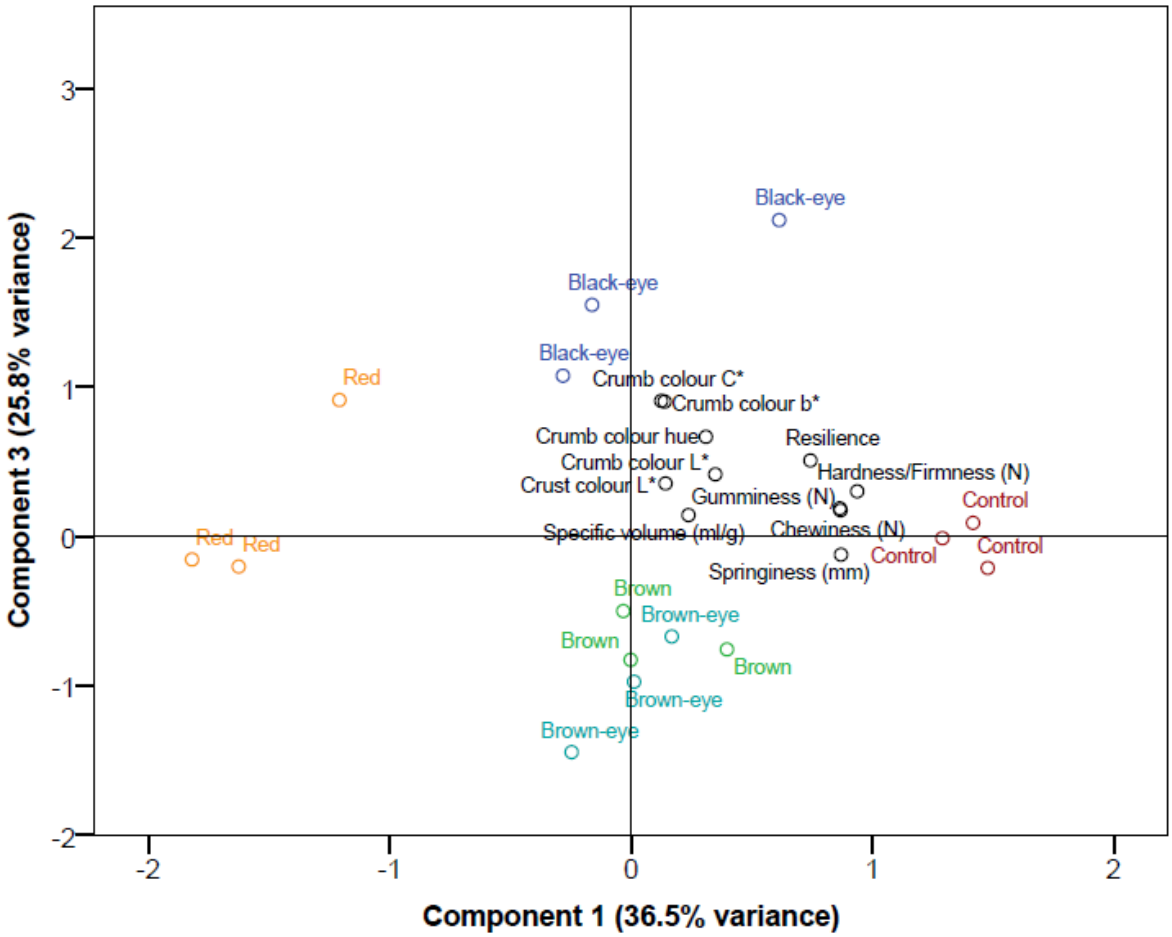


Figure 4.13 Score plot showing differentiation of bread varieties with respect to components 1 and 3.

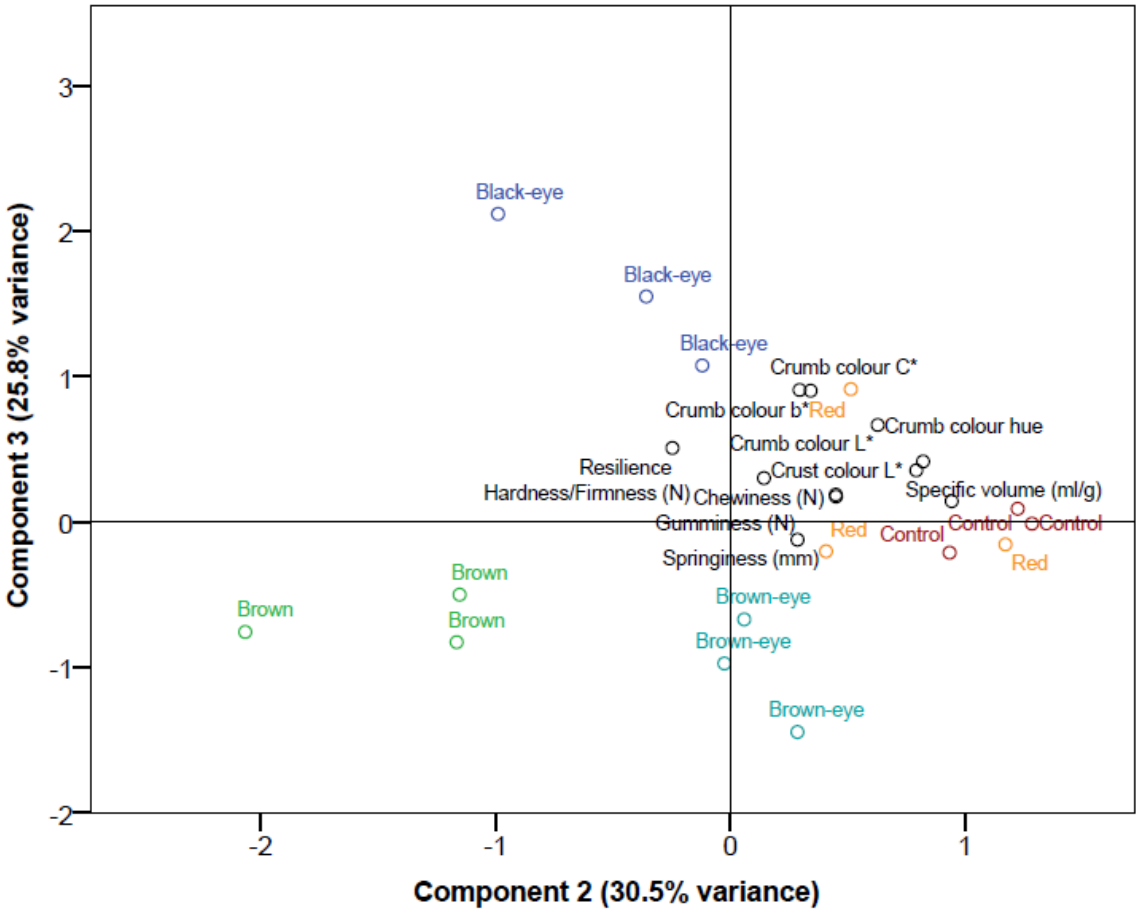


Figure 4.14 Score plot showing differentiation of bread varieties with respect to components 2 and 3.

differentiation could be made between the red and control BGNIF loaves, and differences were depicted between black-eye BGNIF and brown and brown-eye BGNIF loaves.

Bread loaf volume and textural properties are important indicators used in the quality assessment of bread (Różylo & Laskowski, 2011); whilst colour is also an important parameter affecting consumer acceptability of bread (Cappa *et al.*, 2013). The three components (textural properties, specific loaf volume and bread lightness, and crumb colour parameters respectively) identified in the PCA can thus be used as predictors to determine the quality of BGNIF enriched bread.

4.3.4 Sensory evaluation of control white bread and BGNIF enriched breads

The demography of the 36 panellists who partook in the sensory study is as follow: 63.9% females, 77.8% students, 83.3% black people, 80.6% local students, 11.1% < 20 years old, 72.2% within 20 – 29 age group and 8.3% within 30 – 39 and ≥ 40 years age group.

Sensory scores for several sensorial parameters and overall acceptability of white bread supplemented with four BGNIF varieties is presented in Figure 4.15. Based on a score of 3 “neither like nor dislike”, all BGNIF enriched breads were acceptable for the parameters evaluated. Statistically, no significant [$p > 0.05$] difference was perceived by the panellists for the crust colour and texture of the control and BGNIF enriched breads. Crust colour is mainly affected by Maillard and caramelization browning reactions (Anil, 2007), which explains the similar sensory scores as rated for bread crust colour in this study. The similar bread texture scores could be attributed to the favourable texture parameters (as evaluated by texture profile analysis) of the BGNIF enriched breads where crumb firmness, chewiness and gumminess were significantly [$p \leq 0.05$] lower compared to the control bread. The appearance of brown-eye BGNIF bread was rated significantly [$p > 0.05$] similar to that of the control bread, whilst the appearance of black-eye, red and brown BGNIF breads (rated from 3.4 – 3.6) were significantly [$p \leq 0.05$] lower compared to the control bread. No significant [$p > 0.05$] difference was perceived by the panellists between the control, black-eye and brown-eye breads for crumb colour and taste parameters. The perceived crumb colour of BGNIF enriched breads was to a certain extent in agreement with the instrumental colour results, where crumb colour of black-eye and brown-eye breads was found to have high lightness and the colour difference from the control bread was lowest for brown-eye and black-eye breads. Interestingly, crumb colour of brown BGNIF bread and colour difference compared to control bread crumb were not significantly [$p > 0.05$] different from black-eye bread crumb; yet a significant [$p \leq 0.05$] difference was sensorially perceived by the panellists. Aroma of control, red and brown-eye bread loaves were scored in a range of 3.5 – 3.9 with no significant [$p > 0.05$] difference between them; whilst aroma of black-eye and brown BGNIF breads were rated significantly [$p \leq 0.05$] lower compared to the control bread.

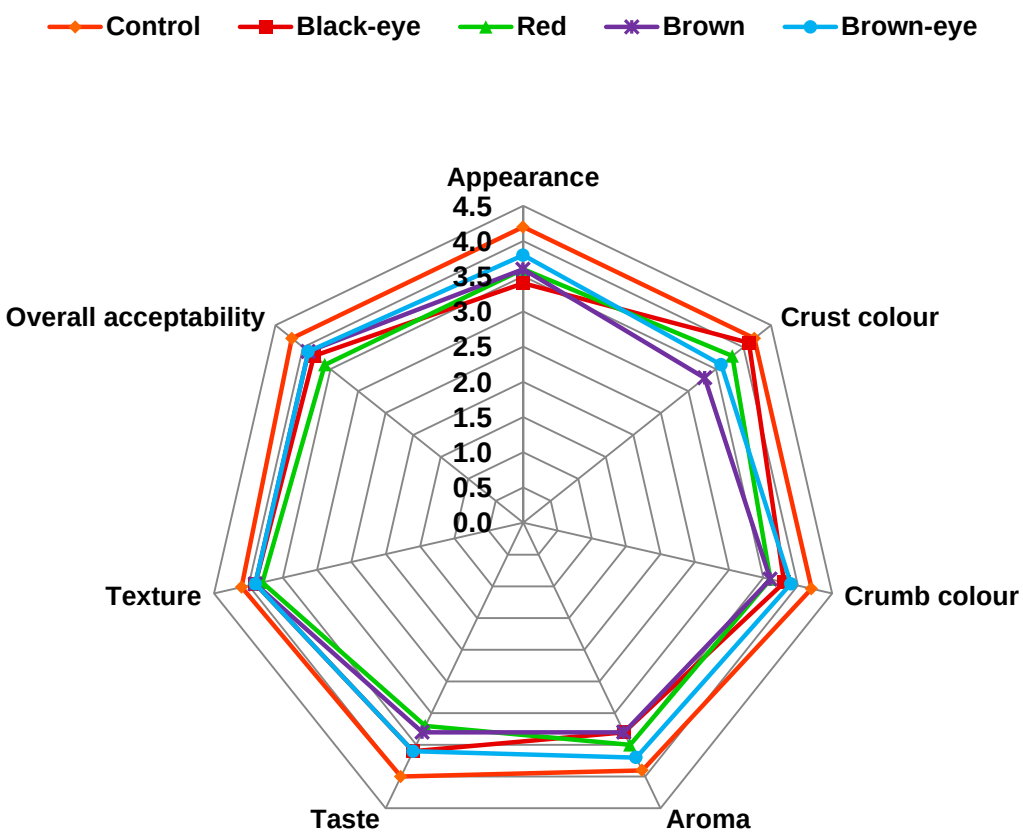


Figure 4.15 Spider plot showing sensory scores of control white bread and Bambara groundnut insoluble dietary fibre enriched breads.
Hedonic rating scale: 1 = Dislike very much, 2 = Dislike moderately,
3 = Neither like nor dislike, 4 = Like moderately, 5 = Like very much.

Overall acceptability of BGNIF enriched breads ranged from 3.6 – 3.9 (like moderately) with black-eye, brown and brown-eye loaves rated significantly [$p > 0.05$] similar to the overall acceptability of the control bread. Based on all sensory parameters, brown-eye BGNIF bread was consistently comparable to the control white bread. Some panellists' comments for this variety included "very nice, sweet and soft/fresh", "typical bread aroma", "this one was the best" and "taste like normal bread".

Categorising panellists according to sensory attributes of the bread loaves

Multivariate analysis of variance indicated that panellists differentiated between BGNIF enriched breads based on significant [$p \leq 0.05$] differences perceived for taste and aroma. To identify the variables for grouping, regression analysis for the identified sensory parameters (taste and aroma) was performed. Regression analysis revealed the independent variables BGNIF, gender and international status of students as significantly [$p \leq 0.05$] impacting the taste parameter. Similarly for aroma, BGNIF, race and international status of the students were identified as significant [$p \leq 0.05$] factors. When applying these data in categorical principal component analysis [CATPCA], it was observed that the variance accounted for by both taste and aroma parameters could be described by two dimensions with eigenvalues greater than one.

For the taste parameter the percentage of variance for dimension 1 was 36.1% and for dimension 2 it was 28.2%, with both dimensions accounting for 64.3% of variance in the optimally scaled variables. The joint category plot (Figure 4.16) shows the relation of independent variables to each other and to the two dimensions. Relating positively to dimension 1 and located close to the centroid for dimension 2, were the black-eye and brown-eye BGNIF breads with taste ratings of "like very much" to "like moderately". Comparably the control bread could be seen as an outlier, indicating the panellists' preference for the brown-eye and black-eye BGNIF enriched breads when comparing the taste of the breads. Dimension 2 was associated with red and brown BGNIF bread that is far removed from neither like nor dislike to dislike very much. Hence, the BGNIF loaves were acceptable to the consumers in taste.

The variation for data describing aroma of the breads was 34.9% for dimension 1 and 33.1% for dimension 2 with both dimensions accounting for 68.0% of variance. From the joint category plot (Figure 4.17) the perceived aroma by the white panellists is a clear outlier which resulted from this group forming the minority panellists, whilst the other variables were located between -3 and 2 for dimension 1 and -1 and 1 for dimension 2. Dimension 1 was closely related to the panellists' preference for aroma of "like moderately" and "like very much" for brown-eye and control breads as preferred by black international students. Dimension 2 was associated with the brown and red BGNIF which was neither liked nor disliked in aroma. Hence, the BGNIF loaves were acceptable to the consumers in aroma.

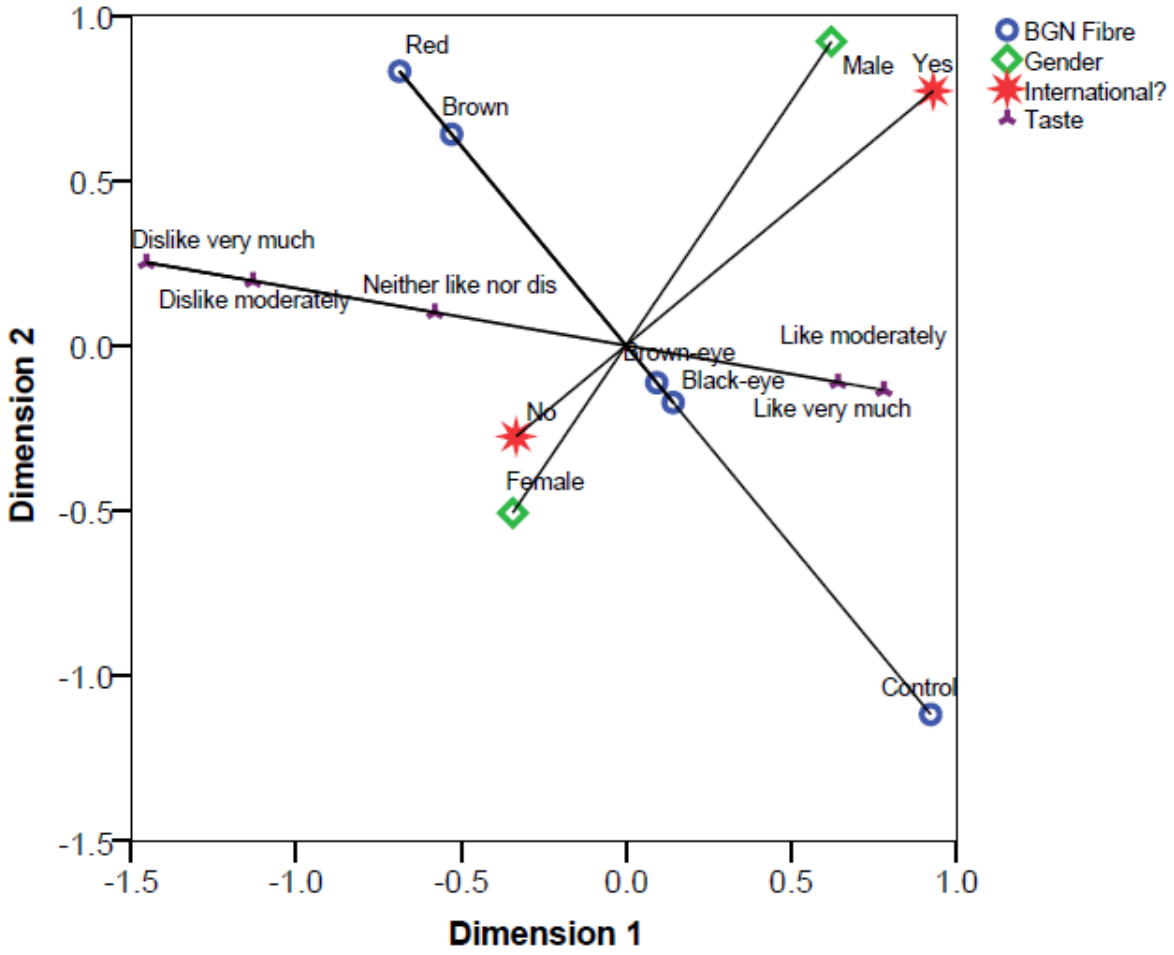


Figure 4.16 Joint category plot for taste preference showing relation of independent variables to each other and their weight to the dimensions.

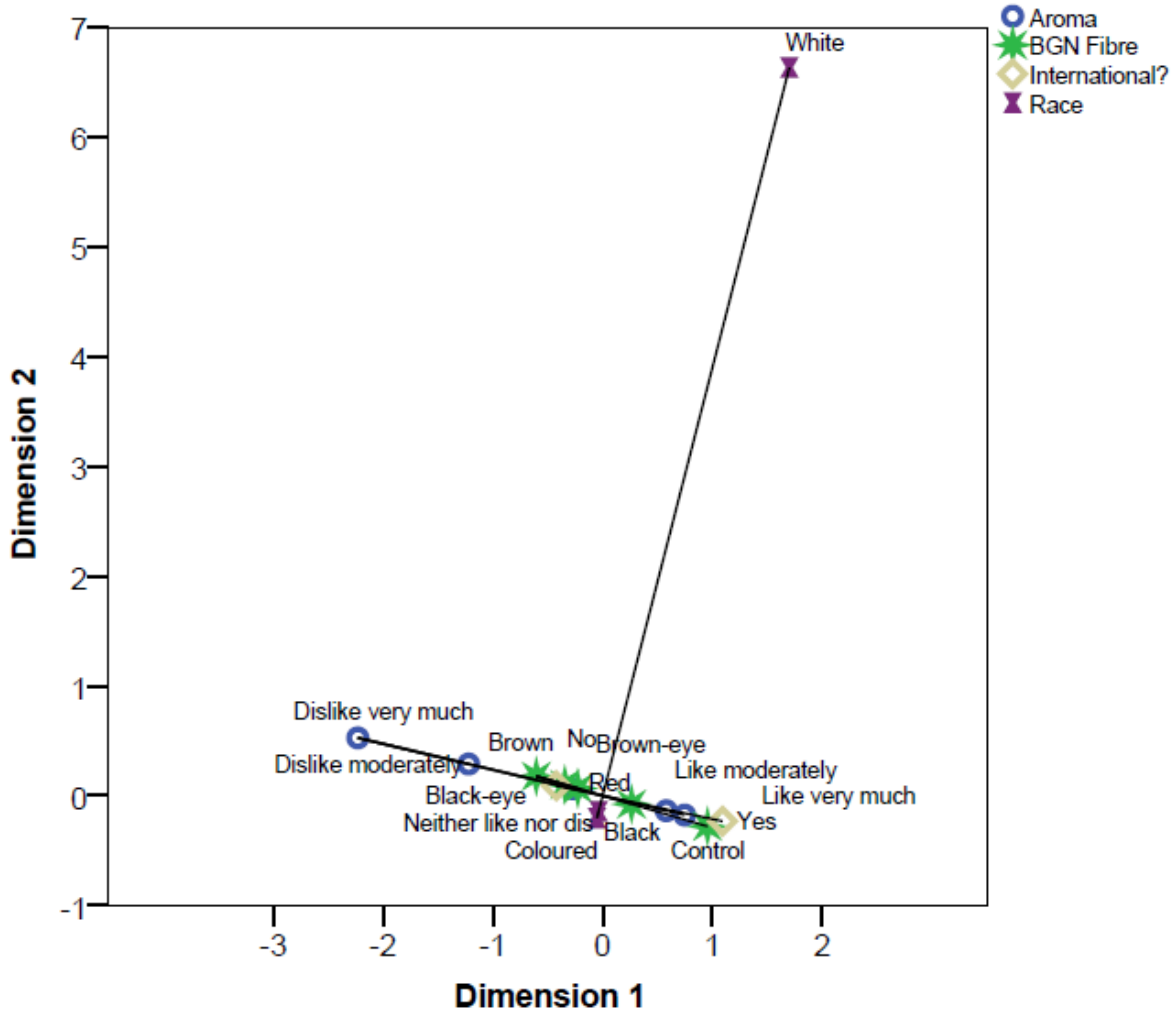


Figure 4.17 Joint category plot for aroma preference showing relation of independent variables to each other and their weight to the dimensions.

By considering these results depicted by CATPCA, all BGNIF enriched loaves were acceptable to the consumers with brown-eye and black-eye BGNIF bread consistently receiving higher ratings. These fibres would thus be favourable for increasing the dietary fibre content of white bread whilst maintaining consumer acceptability of the bread.

4.4 Conclusion

Four varieties of BGNIF (black-eye, red, brown and brown-eye) were successfully incorporated into an optimised white bread formulation. Compared to the control white bread, crumbs of BGNIF enriched loaves contained more pores with small diameters resulting in a uniform grain structure. All BGNIF varieties significantly increased the amount of dietary fibre in the bread, with black-eye and brown-eye having the highest fibre content. As expected all BGNIF enriched breads had lower specific loaf volumes compared to the control bread. Positive textural characteristics were observed in the BGNIF breads, especially the significantly softer crumbs (lower firmness) of the breads compared to the control white bread, which is contrary to results reported in literature. Black-eye and brown-eye bread crumbs were characterised by high lightness and yellowness comparable to the control bread. Similarly, the crust of brown-eye bread was similar in some parameters to the control bread and the colour difference compared to the control was also the lowest for this variety. The objective to determine the functionality of BGNIF's in white bread was thus achieved. The hypothesis that the insoluble fibre from the different BGN varieties will differ in their functionality in white bread is also accepted. All BGNIF enriched breads were positively rated by consumers for all sensorial parameters evaluated. The panellists could not differentiate between texture and crust colour of the control and BGNIF enriched breads. Furthermore, the panellists differentiated between aroma and taste parameters of the bread; with a high preference for the taste of black-eye and brown-eye loaves and for aroma of brown-eye and control loaves. It can be concluded that all BGNIF varieties, but especially black-eye and brown-eye BGNIF varieties, could be successfully added to bread to considerably increase the fibre content of bread with improved physicochemical properties; whilst maintaining consumer acceptability.

References

AACC (2000a). *Approved Methods of the AACC*. Optimized straight-dough bread-making method (Method 10-10.03). St. Paul, Minnesota: American Association of Cereal Chemists.

AACC (2000b). *Approved Methods of the AACC*. Moisture—air-oven methods (Method 44-15.02). St. Paul, Minnesota: American Association of Cereal Chemists.

Abdul-Hamid, A. & Luan, Y.S. (2000). Functional properties of dietary fibre prepared from defatted rice bran. *Food Chemistry*, **68**, 15-19.

Aguilera, J.M. & Germain, J.C. (2007). Advances in image analysis for the study of food microstructure. In: *Understanding and controlling the microstructure of complex foods* (edited by D.J. McClements). p. 270. Cambridge, England: Woodhead Publishing Limited.

Anderson, J.W., Baird, P., Davis Jr., R.H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V. & Williams, C.L. (2009). Health benefits of dietary fibre. *Nutrition Reviews*, **67(4)**, 188-205.

Anil, M. (2007). Using of hazelnut testa as a source of dietary fiber in breadmaking. *Journal of Food Engineering*, **80**, 61-67.

AOAC (2005). Official Method **991.43**. *Official Methods of Analysis of AOAC INTERNATIONAL* 18th Edition. Gaithersburg, MD: AOAC International.

Asami, D.K., Hong, Y.J., Barrett, D.M. & Mitchell, A.E. (2003). Comparison of total phenolic and ascorbic acid content of freeze dried and air dried marionberry, strawberry and corn grown using conventional, organic and sustainable agricultural practices. *Journal of Agricultural and Food Chemistry*, **51**, 1237-1241.

Blackwood, A.D., Salter, J., Dettmar, P.W. & Chaplin, M.F. (2000). Dietary fibre, physicochemical properties and their relationship to health. *The Journal of The Royal Society for the Promotion of Health*, **120**, 242-247.

Blandino, M., Sovrani, V., Marinaccio, F., Reyneri, A., Rolle, L., Giacosa, S., Locatelli, M., Bordiga, M., Travalgia, F., Coïsson, J.D. & Arlorio, M. (2013). Nutritional and technological quality of bread enriched with an intermediated pearled wheat fraction. *Food Chemistry*, **141**, 2549-2557.

Brownlee, I.A. (2011). The physiological roles of dietary fibre. *Food Hydrocolloids*, **25**, 238-250.

Cappa, C., Lucisano, M. & Mariotti, M. (2013). Influence of *Psyllium*, sugar beet fibre and water on gluten-free dough properties and bread quality. *Carbohydrate Polymers*, **98**, 1657-1666.

Cauvain, S.P. (1999). Bread – the product. In: *Technology of Breadmaking* (edited by S.P. Cauvain & L.S. Young). Pp. 11-15. Gaithersburg, Maryland: Aspen Publishers, Inc.

Cauvain, S.P. & Young, L.S. (2006). Key characteristics of existing bakery-product groups and typical variations within such groups. In: *Baked Products: Science, Technology and Practice*. Pp. 16, 22. UK: Blackwell Publishing Ltd.

Chau, C.F. & Huang, Y.L. (2003). Comparison of the chemical composition and physicochemical properties of different fibers prepared from peel of *Citrus sinensis* L. Cv. Liucheng. *Journal of Agricultural and Food Chemistry*, **51**, 2615-2618.

Dalgetty, D.D. & Baik, B. (2006). Fortification of bread with hulls and cotyledon fibers isolated from peas, lentils, and chickpeas. *Cereal Chemistry*, **83(3)**, 269-274.

Demirkesen, I., Kelkar, S., Campanella, O.H., Sumnu, G., Sahin, S. & Okos, M. (2014). Characterization of structure of gluten-free breads by using X-ray microtomography. *Food Hydrocolloids*, **36**, 37-44.

Dhingra, D., Michael, M., Rajput, H. & Patil, R.T. (2012). Dietary fibre in foods: A review. *Journal of Food Science and Technology*, **49(3)**, 255-266.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*, **124**, 411-421.

Eltayeb, A.R.S.M., Ali, A.O., Abou-Arab, A.A. & Abu-Salem, F.M. (2011). Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut (*Vigna subterranea* (L.) Verdc.). *African Journal of Food Science*, **5(2)**, 82-90.

Endress, H. & Fischer, J. (2001). Fibres and fibre blends for individual needs: A physiological and technological approach. In: *Advanced Dietary Fibre Technology* (edited by B.V. McCleary & L. Prosky). Pp. 290-297. UK: Blackwell Science Ltd.

Ferreira, T. & Rasband, W. (2012). ImageJ User Guide. [WWW Document]: URL http://rsbweb.nih.gov/ij/docs/guide/146.html. Accessed on 26 March 2014.

Gómez, M., Ronda, F, Blanco, C.A., Caballero, P.A. & Apesteguía (2003). Effect of dietary fibre on dough rheology and bread quality. *European Food Research & Technology*, **216**, 51-56.

Guillon, F. & Champ, M. (2000). Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International*, **33**, 233-245.

Ishida, N., Takano, H., Naito, S., Isobe, S., Uemura, K., Haishi, T., Kose, K., Koizumi, M. & Kano, H. (2001). Architecture of baked breads depicted by a magnetic resonance imaging. *Magnetic Resonance Imaging*, **19 (6)**, 867-874.

Jeirani, Z., Jan, B.M., Ali, B.S., Noor, I.M., Hwa, S.C. & Saphanuchart, W. (2012). The optimal mixture design of experiments: Alternative method in optimizing the aqueous phase composition of a microemulsion. *Chemometrics and Intelligent Laboratory Systems*, **112**, 1-7.

Jideani, V.A. & Diedericks, C.F. (2013). Dietary fibre from Bambara groundnuts (*Vigna subterranea*). South Africa- Provisional Patent 14 June 2013, 2013/04395.

Jideani, V.A. & Diedericks, C.F. (2014). Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*. In: *Antioxidant-Antidiabetic Agents and Human Health* (edited by O. Oguntibeju). Pp. 187-207. Rijeka, Croatia: Intech Publishers.

Jideani, V.A. & Murevanhema, Y.Y. (2013). A Bambara groundnut milk beverage and probiotic yoghurt produced therefrom and a method for the production thereof. South Africa- Complete Patent 25 January 2013, 2013/00683.

Mondal, A. & Datta, A.K. (2008). Bread baking – A review. *Journal of Food Engineering*, **86**, 465-474.

Polaki, A., Xasapis, P., Fasseas, C., Yanniotis, S. & Mandala, I. (2010). Fiber and hydrocolloid content affect the microstructural and sensory characteristics of fresh and frozen stored bread. *Journal of Food Engineering*, **97**, 1-7.

Ragaee, S., Guzar, I., Dhull, N. & Seetharaman, K. (2011). Effects of fiber addition on antioxidant capacity and nutritional quality of wheat bread. *LWT – Food Science and Technology*, **44**, 2147-2153.

Różylo, R. & Laskowski,J. (2011). Predicting bread quality (bread loaf volume and crumb texture). *Polish Journal of Food and Nutrition Sciences*, **61(1)**, 61-67.

Rupasinghe, H.P.V., Wang, L., Huber, G.M. & Pitts, N.L. (2008). Effect of baking on dietary fibre and phenolics of muffins incorporated with apple skin powder. *Food Chemistry*, **107**, 1217-1224.

Sabanis, D., Lebesi, D. & Tzia, C. (2009). Effect of dietary fibre enrichment on selected properties of gluten-free bread. *LWT – Food Science & Technology*, **42**, 1380-1389.

Sahin, S. & Sumnu, S.G. (2006a). Electromagnetic properties. In: *Physical Properties of Foods*. Pp. 170 – 171. New York: Springer Science+Business Media, LLC.

Sahin, S. & Sumnu, S.G. (2006b). Rheological properties of foods. In: *Physical Properties of Foods*. Pp. 93-95. New York: Springer Science+Business Media, LLC.

Saura-Calixto, F.D. & Bravo, L. (2001). Dietary fiber-associated compounds: Chemistry, analysis, and nutritional effects of polyphenols. In: *Handbook of Dietary Fiber* (edited by S.S. Cho & M.L. Dreher). New York: Marcel Dekker, Inc.

Scanlon, M.G. & Zghal, M.C. (2001). Bread properties and crumb structure. *Food Research International*, **34**, 841-864.

Schaafsma, G. (2004). Health claims, options for dietary fibre. In: *Dietary Fibre* (edited by J.M. van der Kamp, N.G. Asp, J. Miller & G. Schaafsma). Pp. 27-38. The Netherlands: Wageningen Academic Publishers (As cited by Rodríquez *et al.*, 2006).

Schneeman, B.O. (1998). Dietary fibre and gastrointestinal function. *Nutrition Research*, **18**, 625-632.

Stat-Ease (2011). Chapter 2: Triangulating your region of formulation. [WWW Document]: URL http://legacy.statease.com/pubs/MIXprimer.pdf. Accessed on 12 February 2014.

Sudha, M.L., Baskaran, V. & Leelavathi, K. (2007). Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. *Food Chemistry*, **104**, 686-692.

Texture Technologies (2013). Texture profile analysis. [WWW Document]: URL 128.121.92.221/texture_profile_analysis.html. Accessed on 19 December 2013.

Tharanathan, R.N. & Mahadevamma, S. (2003). Grain legumes – a boon to human nutrition. *Trends in Food Science & Technology*, **14**, 507-518.

Tiwari, B.K., Gowen, A. & McKenna, B. (2011). Introduction. In: *Pulse Foods: Processing, Quality and Nutraceutical Applications* (edited by B.K. Tiwari, A. Gowen & B. McKenna). Pp. 1-3. London, UK: Elsevier Inc.

Tosh, S.M. & Yada, S. (2010). Review – Dietary fibres in pulse seeds and fractions: Characterisation, functional attributes, and applications. *Food Research International*, **43**, 450-460.

Wang, J., Rosell, C.M. & Benedito de Barber, C. (2002). Effect of the addition of different fibres on wheat dough performance and bread quality. *Food Chemistry*, **79**, 221-226.

Wang, N. & Toews, R. (2011). Certain physicochemical and functional properties of fibre fractions from pulses. *Food Research International*, **44**, 2515-2523.

Wang, R. & Zhou, W. (2004). Stability of tea catechins in the breadmaking process. *Journal of Agricultural and Food Chemistry*, **52**, 8224-8229.

CHAPTER FIVE

EFFECT OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* (L.) VERDC.) SOLUBLE DIETARY FIBRE ON STABILITY OF ORANGE BEVERAGE EMULSION

Abstract

A 2^2 augmented factorial design was generated to determine the effect of Bambara groundnut soluble dietary fibre [BGNSF] and orange oil at two levels each (15, 30% and 6, 10%, respectively) on the equilibrium backscattering [BS] flux as indicator for emulsion stability. The six emulsion formulations which were obtained were subjected to Turbiscan stability analysis (Turbiscan MA 2000, Formulaction, France) for a scanning period of 1 hr at 10 min intervals. The resulting BS profiles indicated flocculation phenomenon as the major destabilisation mechanism, albeit at low rates for all emulsions. The lowest initial mean BS was measured for formulation 6 (containing 6% orange oil and 30% BGNSF), thus giving an indication of the high stability of this emulsion. Destabilisation kinetics further confirmed this observation with barely any variation in BS observed for formulation 6. All emulsions were characterised by very small decreases in BS, with the maximum equilibrium BS measured as 1.26% for formulation 1 (containing 8% orange oil and 22.5% BGNSF). BGNSF concentration was identified as the impacting factor determining emulsion stability. Formulation 6 containing 6% orange oil and 30% BGNSF was therefore concluded to be the optimal formulation to produce a stable orange beverage emulsion.

5.1 Introduction

Beverage emulsions are a special type of oil-in-water emulsions which are prepared as a concentrate and consumed in diluted form. In both concentrated and diluted forms, a high level of stability should be maintained (Tan, 2004). There are typically two types of beverage emulsions: flavour emulsions which primarily impart aroma and taste to a beverage, and cloud emulsions which impart specific optical properties (i.e. cloudiness) to a beverage (Piorkowski & McClements, 2013). Flavour oils, the main constituent in the oil phase of beverage flavour emulsions, are usually comprised of citrus oils which provide the flavour and some cloud in the beverage. The insolubility of citrus oils in water and their low specific gravity makes it difficult to obtain stable emulsions (Tan, 2004); therefore specific substances known as stabilisers (i.e. texture modifiers, weighting agents, emulsifiers, etc.) with specific physicochemical mechanisms are added to emulsions to make them kinetically stable (Piorkowski & McClements, 2013).

Food hydrocolloids play a key functional role in the preparation and shelf-life control of emulsions. In oil-in-water emulsions most hydrocolloids perform as stabilising agents, whereas only some can perform as emulsifying agents (Dickinson, 2009). As stabilising

agents the hydrocolloids have a thickening, structuring or gelling effect in the aqueous phase, leading to a retardation or prevention of droplet movement and consequently a stable emulsion. Polysaccharide emulsifiers on the other hand stabilises droplets mainly through steric repulsion, as they have a tendency of forming thick hydrophilic interfacial layers (Dickinson, 2009; Piorkowski & McClements, 2013). Gum Arabic (or gum acacia), a natural polysaccharide obtained from trees of the genus *Acacia* (family Leguminosae), is the most widely used polysaccharide emulsifier employed in the formulation of beverage emulsions (Tan, 2004). Despite its excellent emulsification properties in oil-in-water emulsions, some problems have been associated with gum arabic such as its low affinity for oil-water interfaces which means higher concentrations are needed to form stable emulsions, and the unstable supply of consistent high quality gum arabic. A need has therefore been identified for sourcing of alternative hydrocolloids to be used in beverage emulsions (Buffo *et al.*, 2001; Nakamura *et al.*, 2004; Piorkowski & McClements, 2013). Soybean soluble polysaccharide, a soluble fibre fraction obtained from soybean cotyledons, was shown to have good emulsifying properties enabling the formation of stable oil-in-water emulsions. Similar to gum arabic, the soybean soluble polysaccharide's stabilising mechanism is based on a protein fraction which adsorbs at the interface anchoring the hydrophilic carbohydrate fractions which prevent aggregation through steric repulsion (Nakamura *et al.*, 2004, 2006). Apart from their important technological role in emulsions, these polysaccharides which are classified as soluble dietary fibres [SDF] also imparts important physiological functionalities such as the regulation of blood glucose levels and lowering of blood cholesterol (Blackwood *et al.*, 2000). The positive stabilising behaviour of these legume polysaccharide fractions in beverage emulsions opens the gap for further research in the area of legume polysaccharides as sustainable emulsion stabiliser sources.

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) [BGN] is recognised by the Food and Agriculture Organisation [FAO] as one of the eleven primary pulse classes (Tiwari *et al.*, 2011). However, this indigenous African legume is still classified as an underutilised legume seed (Eltayeb *et al.*, 2011). Investigation of the functional properties of BGN flours revealed high emulsion capacity and stability (Aremu *et al.*, 2007; Eltayeb *et al.*, 2011). Jideani & Murevanhema (2013) also successfully produced milk from BGN flour. Considering the high carbohydrate content of the seeds (Bamishaiye *et al.*, 2011), the emulsifying properties exhibited by the BGN flours and the stable BGN milk product could potentially be attributed to the polysaccharide fractions of the seeds. The aim of this study was therefore to evaluate the effect of bambara groundnut soluble dietary fibre [BGNSF] on the stability of an orange oil-in-water beverage emulsion, with a view to highlight the potential of BGNSF as new polysaccharide stabilisers for use in the food industry.

5.2 Materials and Methods

5.2.1 Source of materials

Bambara groundnut soluble dietary fibre [BGNSF] (brown variety) which was isolated from this project was used. Potassium sorbate, sodium benzoate and citric acid (commercial grade, Cape Town, South Africa) were purchased and used in the preparation of the beverage emulsions. Cold pressed orange oil (Puris Natural Aroma Chemicals, South Africa) was used as the oil phase and deionised water was used in the water phase.

5.2.2 Preparation of beverage emulsions

A standardised formula containing 15 – 30% (w/w) BGNSF, 6 – 10% (w/w) orange oil, 0.1% (w/w) sodium benzoate, 0.1% (w/w) potassium sorbate, 0.4% (w/w) citric acid and deionised water were used to prepare the oil-in-water beverage emulsion concentrates. The actual amount of BGNSF and orange oil were determined through a 2^2 factorial design as detailed in section 5.2.3.

Orange beverage emulsions are usually composed of two phases; an oil phase and water phase (Mirhosseini *et al.*, 2008). The water phase was prepared by dispersing sodium benzoate, potassium sorbate and citric acid in deionised water (60°C) by using a high-shear Waring blender (South Africa) and mixing for 2 min at low speed. BGNSF was then gradually added to the water mixture and mixed for 2 min at high speed, followed by the gradual addition of the orange oil and further mixing at high speed for 2 min. The initial coarse emulsion was then subjected to homogenisation at 12000 rpm for 5 min by an Ultra Turrax homogeniser (IKA T25 digital, Germany) to obtain fine emulsification. Emulsion microstructure and kinetic stability was evaluated immediately after homogenisation.

5.2.3 Experimental design for emulsion optimisation

A 2^2 augmented factorial design (two factors each at two levels) was used for optimisation of an orange beverage emulsion formulation. The Design-Expert (Stat-Ease Inc., USA, version 8.0.7, 2010) statistical software was used to generate the factorial design. The two independent variables BGNSF and orange oil were varied at two levels (as shown in Table 5.1) and their effects determined on the equilibrium backscattering (emulsion stability indicator). A total of six formulations were tested, including a replication of the centre points (Table 5.2). The emulsions were prepared in randomised order according to the procedure specified in section 5.2.2. Emulsion stability was measured as detailed in section 5.2.4 (page 132).

Table 5.1 Independent variables and their levels as established with 2^2 factorial design[1,2]

		Coded variable levels (x_i)		
Variable	**Symbol**	**Low (-1)**	**Centre (0)**	**High (+1)**
Orange oil (% w/w)	X_1	6	8	10
BGNSF (% w/w)	X_2	15	22.5	30

[1] BGNSF: Bambara groundnut soluble dietary fibre.
[2] Transformation from coded (x_i) to the uncoded (X_i) values is given by $2x_1 + 8$ and $7.5x_2 + 22.5$, respectively.

Table 5.2 Matrix of the 2^2 factorial design[1,2]

	Independent variables	
Run	**Orange oil (X_1, % w/w)**	**BGNSF (X_2, % w/w)**
1	8 (0)	22.5 (0)
2	8 (0)	22.5 (0)
3	10 (+1)	15 (-1)
4	10 (+1)	30 (+1)
5	6 (-1)	15 (-1)
6	6 (-1)	30 (+1)

[1] Coded values indicated in brackets: -1, 0 and +1 represents low levels, centre points and high levels respectively.
[2] BGNSF: Bambara groundnut soluble dietary fibre.

5.2.4 Emulsion stability evaluation

The six formulations were subjected to Turbiscan stability analysis (Turbiscan MA 2000, Formulaction, France) to determine the most stable and thus optimal beverage emulsion formulation. This optical scanning analyser is used for analysis of the physical destabilisation of concentrated emulsions and other liquid dispersions (Mengual *et al.*, 1999). A sample volume of 7 ml was filled into a Turbiscan tube (flat-bottomed cylindrical glass tube: length 65 mm) and immediately placed into the instrument for measurement. Each sample was scanned along their height (from bottom to top) for a period of 60 min at 10 min intervals. Briefly, as described by Mengual *et al.* (1999), the measurement principle of the Turbiscan is based on the backscattering [BS] and transmission of light received through two synchronous detectors. The light going through the sample (0°) is received by the transmission detector, and the light scattered backward by the sample (135°) is received by the backscattering detector. The backscattering and/or transmission profiles allow for detection of two major destabilisation mechanisms: particle size variation (i.e. flocculation, coalescence) and particle migration (i.e. creaming, sedimentation). Creaming phenomenon is observed as a peak in delta BS curves between 0 – 20 mm, whereas the flocculation phenomenon is observed in the optimum zone of 20 – 50 mm (a zone not affected by creaming) by measurement of the average backscattering as a function of storage time (Àlvarez Cerimedo *et al.*, 2010). The mechanism responsible for emulsion instability is thus accurately determined without disrupting the original system; consequently the formulation yielding the most stable emulsion could be identified.

5.2.5 Emulsion microstructure

The microstructure of each emulsion was evaluated immediately after homogenisation by using a Ken-a-Vision TU-19542C (Ken-a-Vision Mfg Co. Inc., USA) digital microscope. A drop of each beverage emulsion was placed on a microscope slide and covered with a cover-slip. The emulsions were observed with a 100x magnification (10x ocular and 10x objective) and images recorded with Applied Vision 4 software (Ken-a-Vision Mfg Co. Inc., version 4.1.12, USA).

5.3 Results and Discussion

5.3.1 Optimal beverage emulsion

The optimal emulsion formulation was identified based on the highest stability as reported in section 5.3.2 (page 134). Images of the emulsions evaluated are shown in Figure 5.1.

Figure 5.1 Six optimisation formulations as determined by a 2^2 augmented factorial design. **(1)** 8% oil, 22.5% BGNSF; **(2)** 8% oil, 22.5% BGNSF; **(3)** 10% oil, 15% BGNSF; **(4)** 10% oil, 30% BGNSF; **(5)** 6% oil, 15% BGNSF; **(6)** 6% oil, 30% BGNSF.
BGNSF: Bambara groundnut soluble dietary fibre.

5.3.2 Beverage emulsion stability

Since all emulsion concentrates were opaque during measurement, only the backscattering profiles were considered for stability evaluation. The BS profile (and corresponding reference mode) of each emulsion is shown in Figures 5.2 to 5.7. The BS initial mean values along the entire tube were 67.79%, 68.62%, 77.22%, 69.94%, 67.79% and 62.99% for formulations 1 to 6, respectively. Formulation 3 (10% oil, 15% BGNSF) was characterised by the highest initial mean BS whereas formulation 6 (6% oil, 30% BGNSF) had the lowest initial mean BS. As noted by Àlvarez Cerimedo *et al.* (2010), the initial mean BS is greatly dependent on the mean particle diameter, and a lower BS correlates to a smaller volume-weighted mean diameter ($D_{4,3}$). The authors also noted that emulsions with the lowest $D_{4,3}$ had the highest stability. This observation gives a preliminary indication that formulation 6 would yield the highest emulsion stability; although the factor affecting stability is not yet clear at this stage since both orange oil and BGNSF were varied.

From the BS profiles it is also observed that the main mechanism of destabilisation for all emulsions was flocculation, without consideration of the region below 8 mm and 63 mm which represents the metal base and beginning of the samples free surface, respectively (Juliano *et al.*, 2011). Flocculation was evident by the decrease in mean BS (Àlvarez Cerimedo *et al.*, 2010). The rate of destabilisation was very slow in all emulsions; and in formulations with the highest BGNSF concentrations (irrespective of the oil concentration, i.e. formulation 4 and formulation 6 containing 30% BGNSF) barely any flocculation was observed. A stable emulsion was characterised by BS profiles which were overlaid on one curve (Formulaction, 2009), thus the BS% of formulation 6 (6% orange oil, 30% BGNSF) which remained unchanged clearly shows the high stability of this emulsion. To quantify the stability of each emulsion and determine which variable influenced stability, destabilisation kinetics was evaluated for each emulsion as described in the next section- flocculation quantification.

Flocculation quantification of beverage emulsions

The variations in BS data as observed in the zone characterised by flocculation is shown in Figure 5.8 as a function of destabilisation kinetics (ΔBS flux) of each emulsion over time. This graph clearly indicates the stability of the different emulsion formulations and also shows which independent variable predominantly influences stability. Emulsion formulation 1 (8% orange oil, 22.5% BGNSF) which was one of the centre point formulations showed the greatest destabilisation with an almost linear decrease in BS of 1.26%. Interestingly, formulation 2 (8% orange oil, 22.5% BGNSF) which was the replicate of the centre points destabilised at a lower rate with a decrease in BS of 0.55%; this formulation was also observed as the third most stable emulsion. Formulation 3 (10% orange oil, 15% BGNSF)

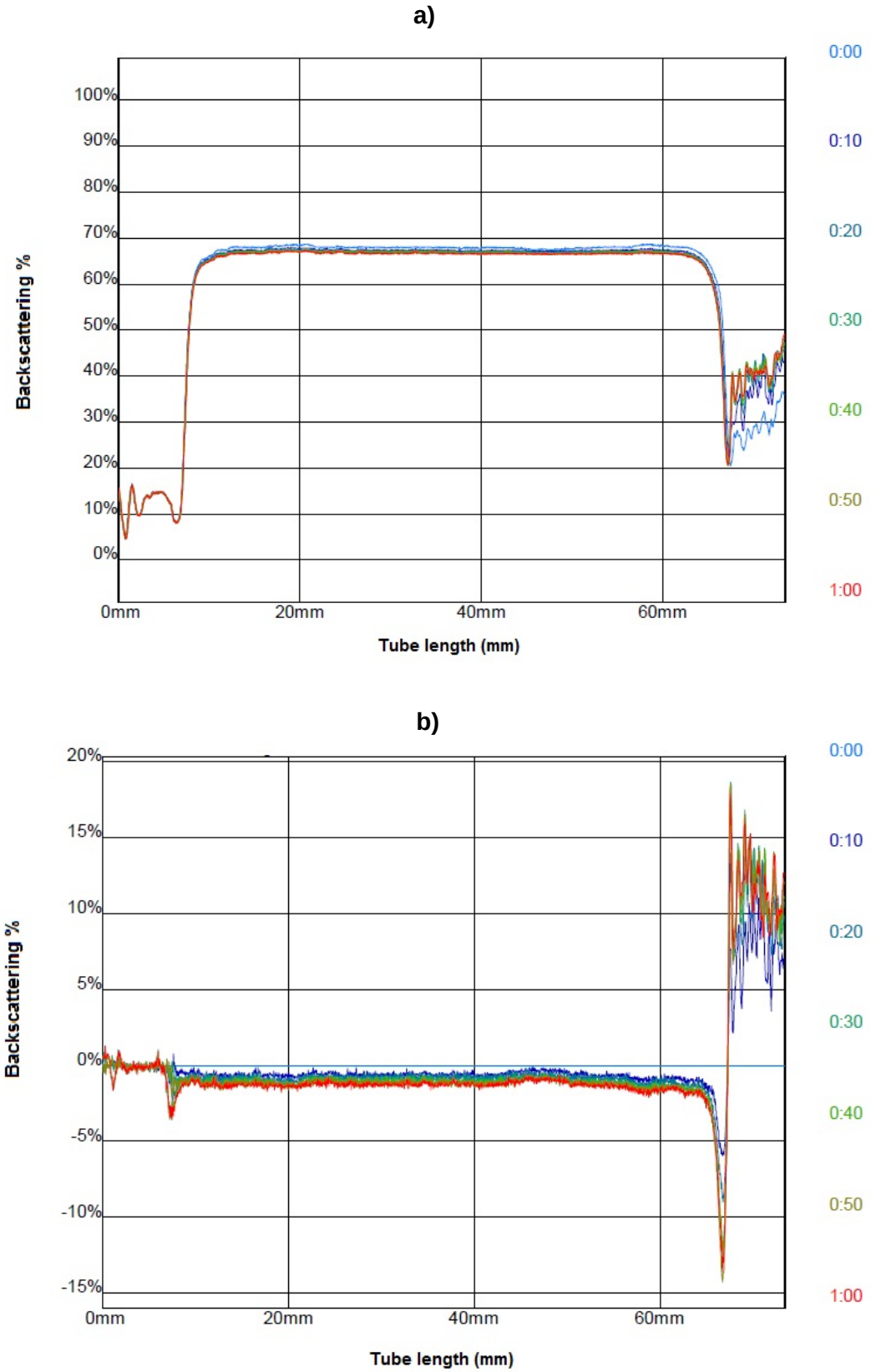
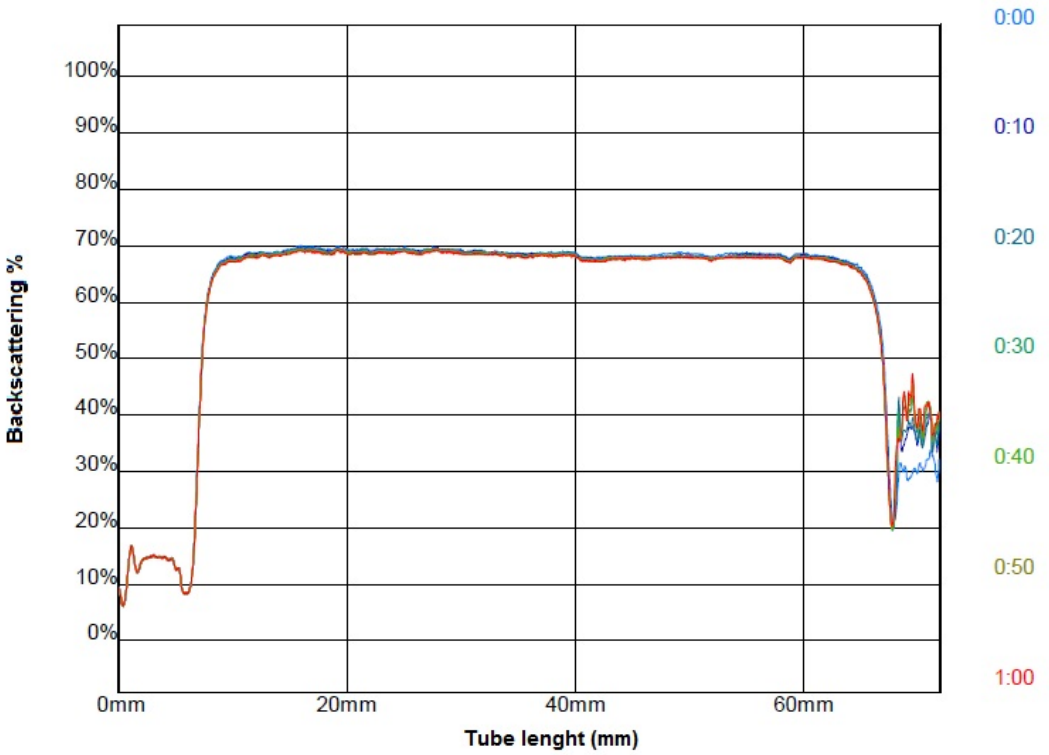


Figure 5.2 **(a)** Backscattering profile and **(b)** backscattering reference mode profile of emulsion formulation 1 (8% orange oil, 22.5% BGNSF) represented as a function of storage time and tube length.
BGNSF: Bambara groundnut soluble dietary fibre.

a)



b)

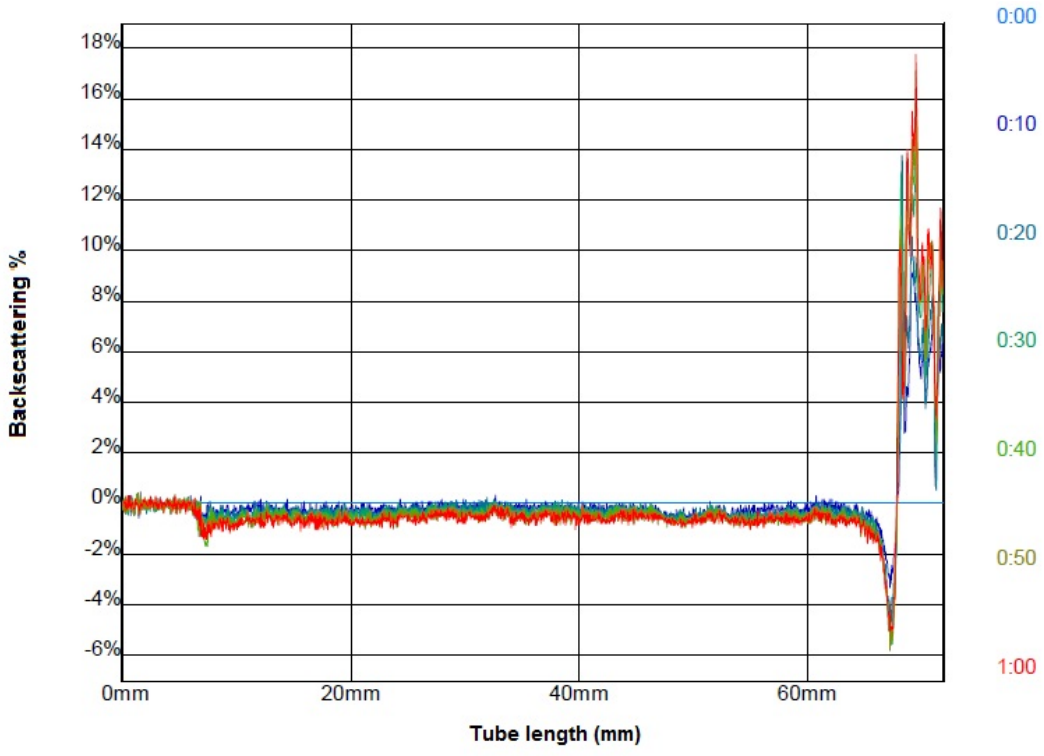


Figure 5.3 **(a)** Backscattering profile and **(b)** backscattering reference mode profile of emulsion formulation 2 (8% orange oil, 22.5% BGNSF) represented as a function of storage time and tube length.
BGNSF: Bambara groundnut soluble dietary fibre.

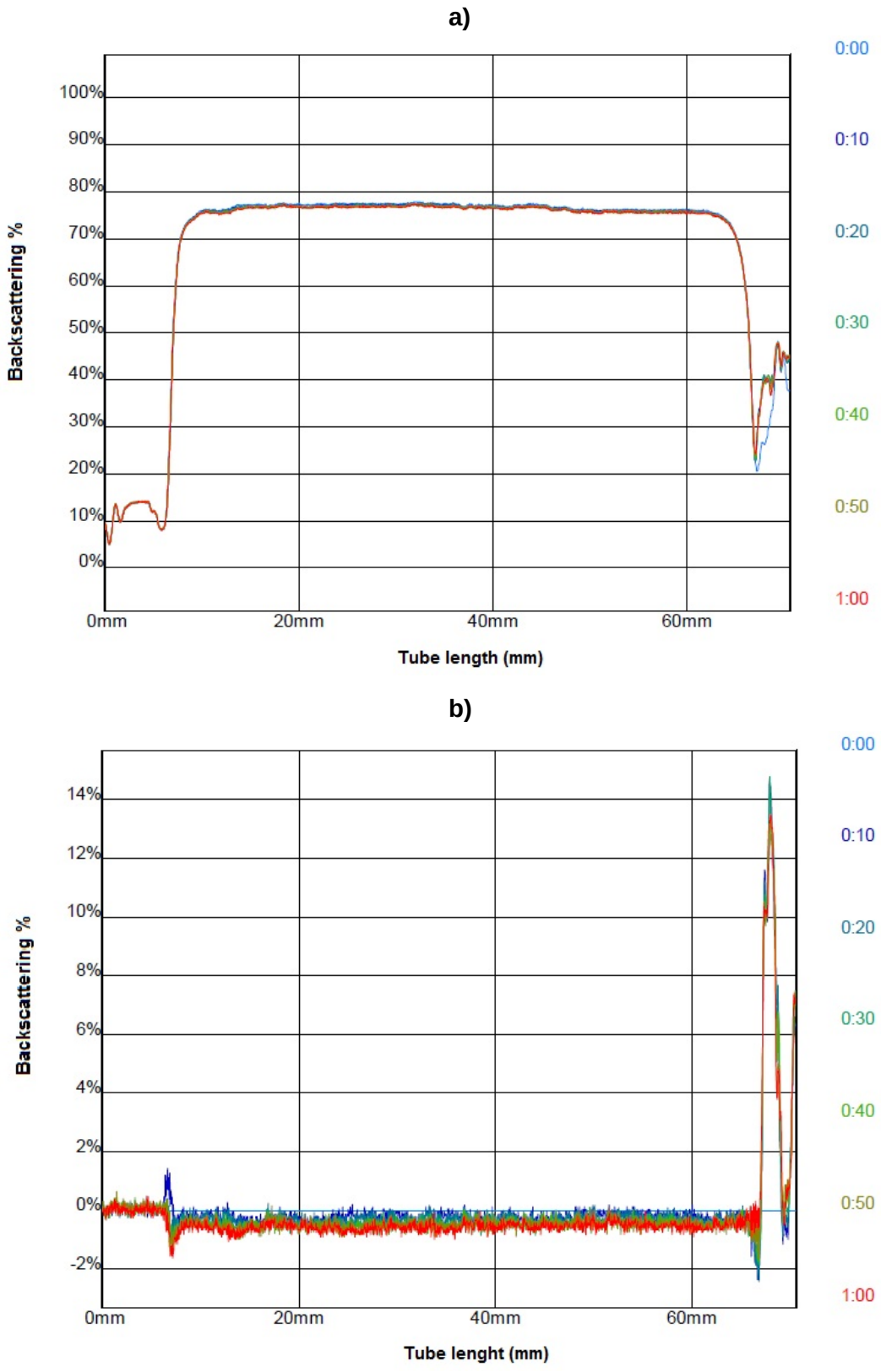


Figure 5.4 **(a)** Backscattering profile and **(b)** backscattering reference mode profile of emulsion formulation 3 (10% orange oil, 15% BGNSF) represented as a function of storage time and tube length.
BGNSF: Bambara groundnut soluble dietary fibre.

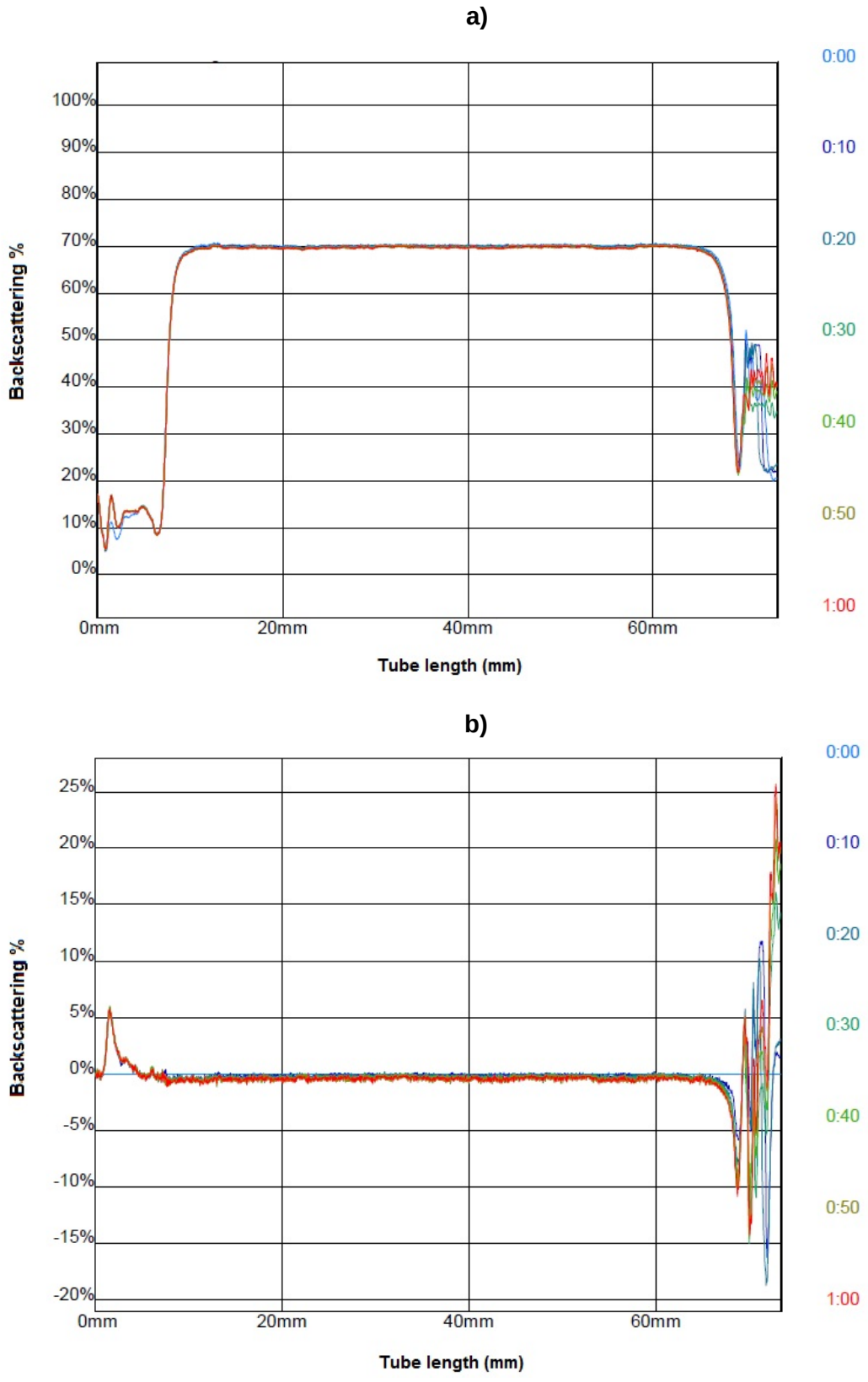


Figure 5.5 **(a)** Backscattering profile and **(b)** backscattering reference mode profile of emulsion formulation 4 (10% orange oil, 30% BGNSF) represented as a function of storage time and tube length.
BGNSF: Bambara groundnut soluble dietary fibre.

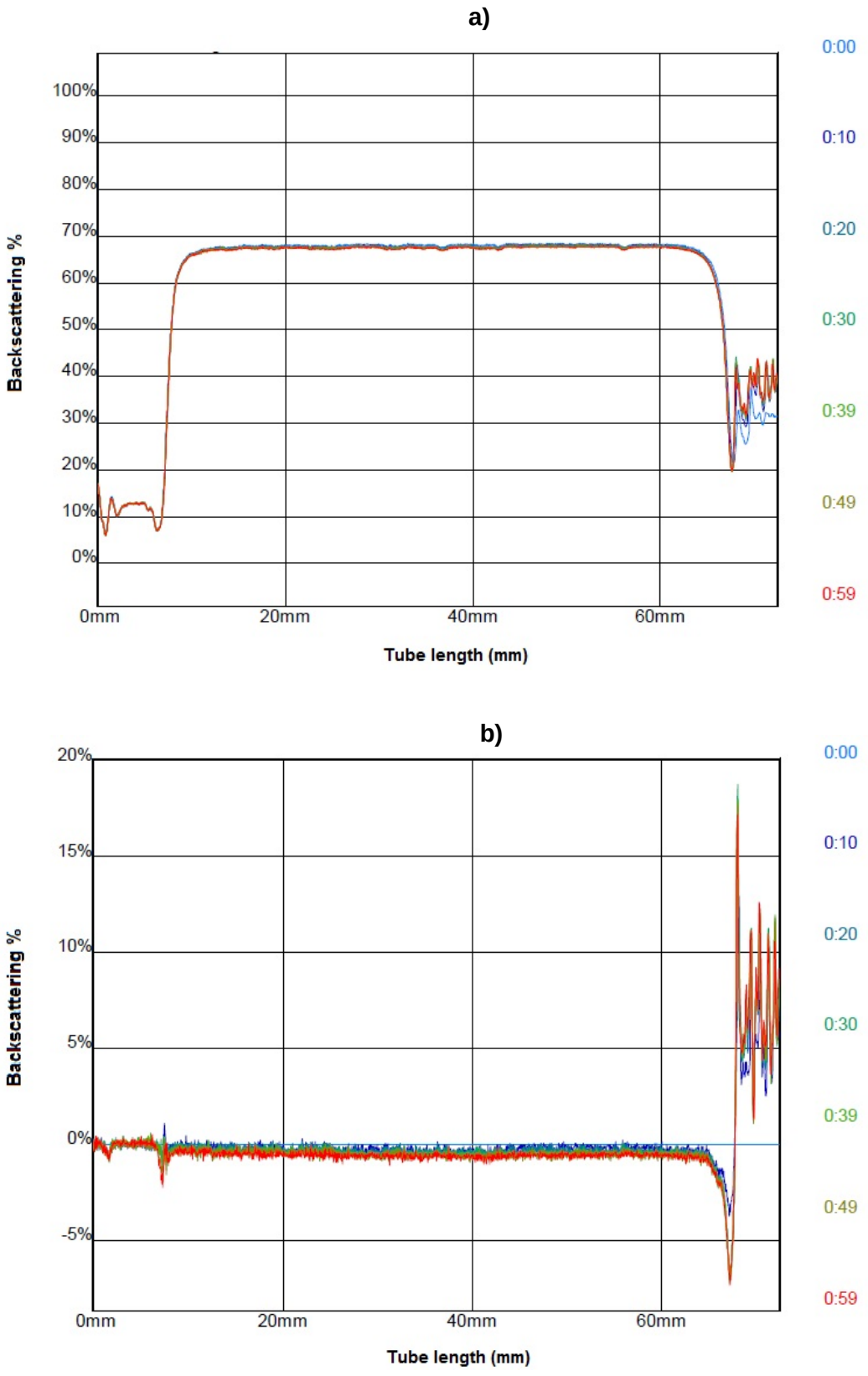


Figure 5.6 **(a)** Backscattering profile and **(b)** backscattering reference mode profile of emulsion formulation 5 (6% orange oil, 15% BGNSF) represented as a function of storage time and tube length.
BGNSF: Bambara groundnut soluble dietary fibre.

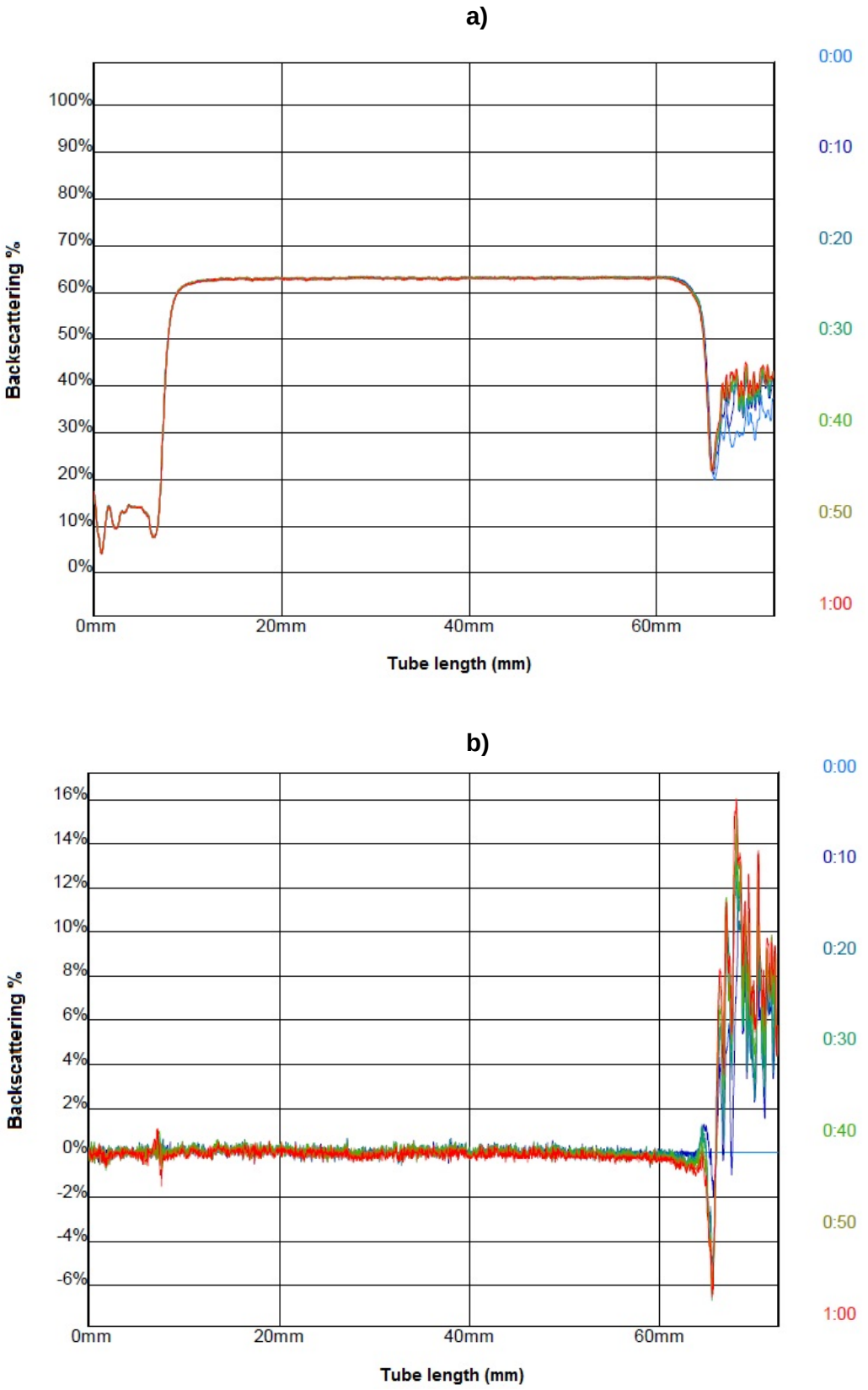


Figure 5.7 **(a)** Backscattering profile and **(b)** backscattering reference mode profile of emulsion formulation 6 (6% orange oil, 30% BGNSF) represented as a function of storage time and tube length.
BGNSF: Bambara groundnut soluble dietary fibre.

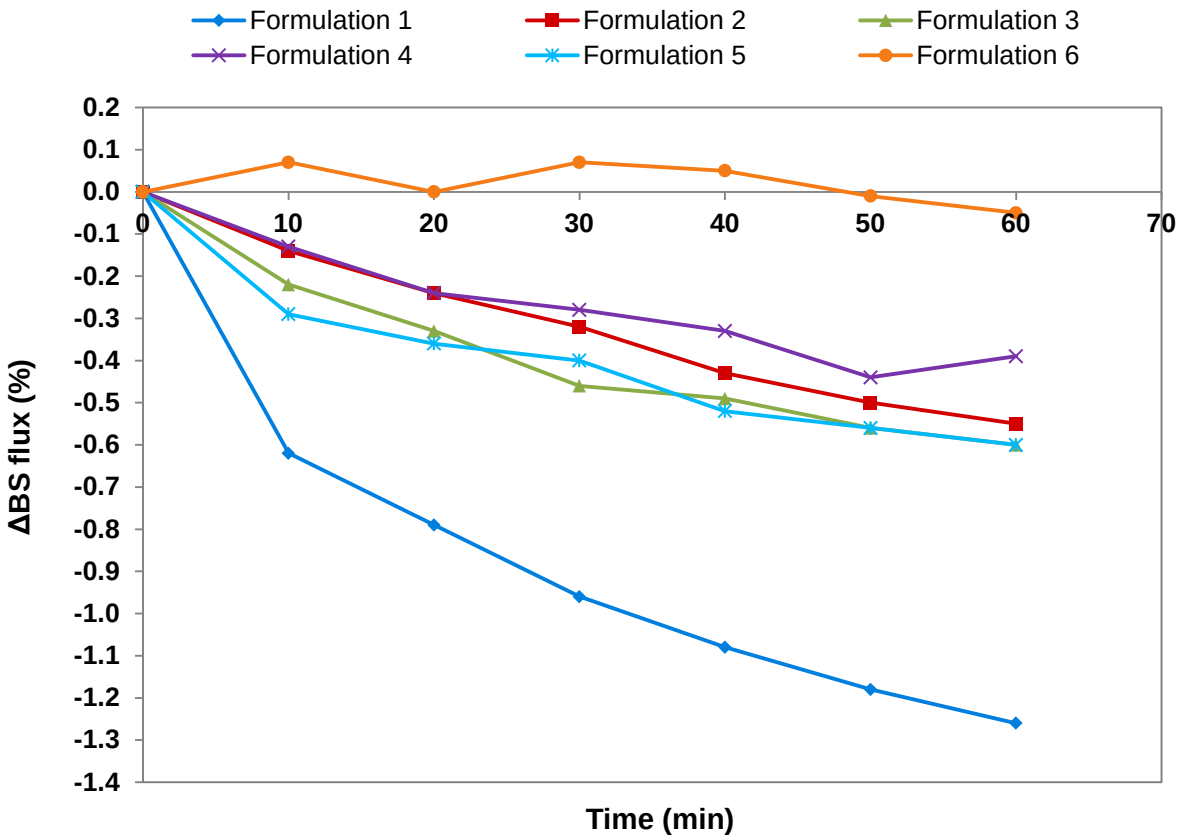


Figure 5.8 Variations in delta backscattering flux of the six optimisation formulations, measured over a storage period of 60 min. Formulations composition: **(1)** 8% oil, 22.5% BGNSF; **(2)** 8% oil, 22.5% BGNSF; **(3)** 10% oil, 15% BGNSF; **(4)** 10% oil, 30% BGNSF; **(5)** 6% oil, 15% BGNSF; **(6)** 6% oil, 30% BGNSF. BGNSF: Bambara groundnut soluble dietary fibre.

and formulation 5 (6% orange oil, 15% BGNSF) showed similar destabilisation behaviour and had a decrease in BS of 0.6%. Formulation 4 (10% orange oil, 30% BGNSF) had a small decrease in BS (0.39%), whereas formulation 6 (6% orange oil, 30% BGNSF) remained fairly constant with a decrease in BS of almost zero (0.05%). The effect of BGNSF concentration on emulsion stability is clearly seen in all formulations. Increase in BGNSF concentration led to higher emulsion stability, irrespective of a low/high orange oil concentration. Formulation 6 was thus identified as the optimal formulation with the highest stability. This observation was further confirmed by the equilibrium BS as shown in Table 5.3, where BS for formulation 6 showed almost no variation with time. Formulations 1 and 2 which represent the centre points differed highly in their equilibrium BS. When considering the observation of increased emulsion stability with increasing BGNSF concentration, formulation 2 correlates to this observation and formulation 1 could thus be considered an outlier.

5.3.3 Microstructure

The microstructure of all emulsions is shown in Figure 5.9. The micrographs showed that the oil droplets for all emulsions were dispersed in the water phase. No distinct size variation could be observed and all emulsions seemingly had droplet distributions of homogenous nature. The droplets in all emulsions were relatively small making it difficult to clearly observe the individual droplets; however it was noted that the emulsions were non-flocculated since flocs are fairly large and could possibly be detected in concentrated systems (McClements, 2007). The apparent absence of flocs is in agreement with the stability of the emulsions as determined by turbiscan stability analysis.

5.4 Conclusion

Turbiscan stability analysis was successfully applied to identify the destabilisation mechanism in the six optimisation formulations tested. Initial mean backscattering data indicated that the formulation containing the highest concentration BGNSF and lowest concentration oil would yield the most stable emulsion; which was further confirmed by the backscattering profiles. Furthermore backscattering kinetics clearly showed that BGNSF concentration determined the stability of each emulsion. The objective to establish the ability of BGNSF on stabilising a beverage emulsion was thus achieved, and the hypothesis that BGNSF will stabilise a beverage emulsion is accepted. The optimal orange oil-in-water beverage emulsion was identified as the emulsion containing 30% brown BGNSF and 6% orange oil. These results positively highlight the potential of bambara groundnut soluble fibre as polysaccharide stabiliser for use in oil-in-water emulsions.

Table 5.3 Equilibrium (eq.) backscattering response for each emulsion formulation[1]

Formulation	Independent variables		Dependent variable
	Orange oil (X_1, % w/w)	**BGNSF (X_2, % w/w)**	**Eq. backscattering (% BS at 1 hr)**
1	8 (0)	22.5 (0)	-1.26
2	8 (0)	22.5 (0)	-0.55
3	10 (+1)	15 (-1)	-0.60
4	10 (+1)	30 (+1)	-0.39
5	6 (-1)	15 (-1)	-0.60
6	6 (-1)	30 (+1)	-0.05

[1] BGNSF: Bambara groundnut soluble dietary fibre; BS: backscattering.

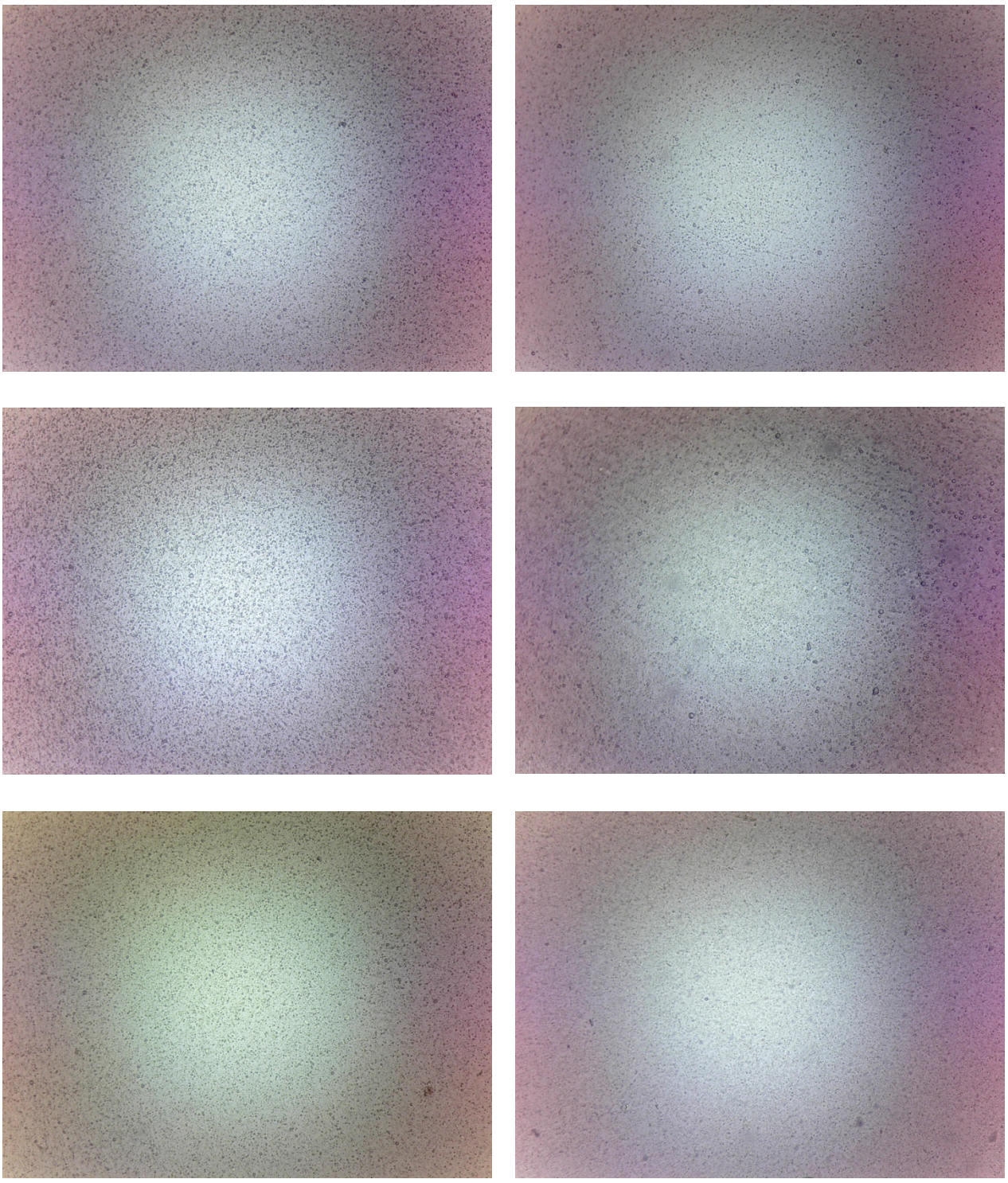


Figure 5.9 Micrographs of the six optimisation beverage emulsions (diluted with distilled water 1:3). Formulations composition: **(1)** 8% oil, 22.5% BGNSF; **(2)** 8% oil, 22.5% BGNSF; **(3)** 10% oil, 15% BGNSF; **(4)** 10% oil, 30% BGNSF; **(5)** 6% oil, 15% BGNSF; **(6)** 6% oil, 30% BGNSF.
BGNSF: Bambara groundnut soluble dietary fibre.

Further research is recommended to establish the mechanism of stabilisation by BGNSF and also the effects of the different varieties on emulsion stability.

References

Àlvarez Cerimedo, M.S., Iriart, C.H., Candal, R.J. & Herrera, M.L. (2010). Stability of emulsions formulated with high concentrations of sodium caseinate and trehalose. *Food Research International*, **43**, 1482-1493.

Aremu, M.O., Olaofe, O. & Akintayo, E.T. (2007). Functional properties of some Nigerian varieties of legume seed flours and flour concentration effect on foaming and gelation properties. *Journal of Food Technology*, **5(2)**, 109-115.

Bamishaiye, O.M., Adegbola, J.A. & Bamishaiye, E.I. (2011). Bambara groundnut: an under-utilized nut in Africa. *Advances in Agricultural Biotechnology*, **1**, 60-72.

Blackwood, A.D., Salter, J., Dettmar, P.W. & Chaplin, M.F. (2000). Dietary fibre, physicochemical properties and their relationship to health. *The Journal of The Royal Society for the Promotion of Health*, **120**, 242-247.

Buffo, R.A., Reineccius, G.A. & Oehlert, G.W. (2001). Factors affecting the emulsifying and rheological properties of gum acacia in beverage emulsions. *Food Hydrocolloids*, **15**, 53-66.

Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids*, **23**, 1473-1482.

Eltayeb, A.R.S.M., Ali, A.O., Abou-Arab, A.A. & Abu-Salem, F.M. (2011). Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut (*Vigna subterranea* (L.) Verdc.). *African Journal of Food Science*, **5(2)**, 82-90.

Formulaction (2009). Stability of various beverage emulsions. Application paper. Pp. 1-7. [WWW Document]: URL http://www.formulaction.com/publication-stability.html. Accessed on 22 November 2013.

Jideani, V.A. & Murevanhema, Y.Y. (2013). A Bambara groundnut milk beverage and probiotic yoghurt produced therefrom and a method for the production thereof. South Africa- Complete Patent 25 January 2013, 2013/00683.

Juliano, P., Kutter, A., Cheng, L.J., Swiergon, P., Mawson, R. & Augustin, M.A. (2011). Enhanced creaming of milk fat globules in milk emulsions by the application of ultrasound and detection by means of optical methods. *Ultrasonics Sonochemistry*, **18**, 963-973.

McClements, D.J. (2007). Critical review of techniques and methodologies for characterization of emulsion stability. *Critical Reviews in Food Science and Nutrition*, **47**, 611-649.

Mengual, O., Meunier, G., Cayré, I., Puech, K. & Snabre, P. (1999). Turbiscan MA 2000: multiple light scattering measurement for concentrated emulsion and suspension instability analysis. *Talanta*, **50**, 445-456.

Mirhosseini, H., Tan, C.P., Hamid, N.S.A. & Yusof, S. (2008). Optimization of the contents of arabic gum, xanthan gum and orange oil affecting turbidity, average particle size, polydispersity index and density in orange beverage emulsion. *Food Hydrocolloids*, **22**, 1212-1223.

Nakamura, A., Takahashi, T., Yoshida, R., Maeda, H. & Corredig, M. (2004). Emulsifying properties of soybean soluble polysaccharide. *Food Hydrocolloids*, **18**, 795-803.

Nakamura, A., Yoshida, R., Maeda, H. & Corredig, M. (2006). Soy soluble polysaccharide stabilization at oil-water interfaces. *Food Hydrocolloids*, **20**, 277-283.

Piorkowski, D.T. & McClements, D.J. (2013). Review – Beverage emulsions: Recent developments in formulation, production and applications. *Food Hydrocolloids*, doi: 10.1016/j.foodhyd.2013.07.009.

Tan, C. (2004). Beverage emulsions. In: *Food Emulsions 4th Edition, Revised and Expanded* (edited by S.E. Friberg, K. Larsson & J. Sjöblom). Pp. 485-524. New York, USA: Marcel Dekker, Inc.

Tiwari, B.K., Gowen, A. & McKenna, B. (2011). Introduction. In: *Pulse Foods: Processing, Quality and Nutraceutical Applications* (edited by B.K. Tiwari, A. Gowen & B. McKenna). Pp. 1-3. London, UK: Elsevier Inc.

CHAPTER SIX
SUMMARY AND CONCLUSIONS

Bambara groundnut [BGN] is an underutilised legume with great nutritional and technological potential. To highlight the importance of this crop, the following objectives were identified in this study:

1. Isolation and characterisation of the insoluble and soluble dietary fibre fractions from BGN with a view to establish their potential for use in food applications.
2. Incorporation of BGN insoluble dietary fibre [BGNIF] into white bread formulation, with a view to highlight the potential of BGNIF varieties as new fibre sources for use in bread and potentially other food formulations.
3. Evaluation of BGN soluble dietary fibre [BGNSF] on the stability of an orange oil-in-water beverage emulsion, with a view to highlight the potential of BGNSF as new polysaccharide stabilisers for use in the food industry.

The first objective was achieved as both BGNIF and BGNSF were extracted (extraction via enzymatic treatment, followed by centrifugation and diafiltration to obtain the insoluble and soluble fractions respectively) from four (black-eye, red, brown and brown-eye) varieties, and consequently characterised for several physicochemical and functional properties. Important characteristics include the high lightness of black-eye BGNIF and all BGNSF varieties which are important for incorporation of fibres into bakery products. All fibres were characterised by high bulk density (compared to lower bulk density of other pulse cotyledon fibres) which is beneficial when considering packaging costs. The highest polyphenol content of black-eye and red BGNSF indicates the possible antioxidant activity of these fibres. The major neutral sugars in BGNIF varieties were the co-eluted arabinose/galactose, xylose and mannose, with proportions of these monomers indicative of hemicellulosic polysaccharides. Xylose and mannose were also the major constituents of BGNSF variants, and together with galactose were indicative of galactomannans which are important ingredients used in beverages. Important hydration properties were the higher swelling capacity of BGNIF's compared to commercial fibres, whilst black-eye BGNIF had a swelling capacity comparable to apple fibre. Compared to commercial pea fibres all BGN fibres, but especially BGNSF's, had higher fat absorption capacity. This is an important characteristic when considering inclusion of such fibres in high fat products and emulsions, where a stabilising effect will be achieved.

The second objective was achieved by incorporating all four BGNIF's (black-eye, red, brown and brown-eye) into an optimal white bread formulation. BGNIF's imparted positive characteristics to bread such as the uniform crumb grain structure and smaller pores, and the higher total dietary fibre content compared to the control bread. Positive textural characteristics were the reduced gumminess, chewiness and softer crumbs of BGNIF

breads. Black-eye and brown-eye bread crumbs were characterised by high lightness and yellowness comparable to the control bread. All BGNIF enriched breads were positively accepted by consumers for all sensorial parameters evaluated. The panellists could not differentiate between texture and crust colour of the control and BGNIF enriched breads. Furthermore, the panellists differentiated between aroma and taste parameters of the bread; with a high preference for the taste of black-eye and brown-eye loaves and for aroma of brown-eye and control loaves.

The third objective was also attained, as it was shown that BGNSF was effective in stabilising an orange beverage emulsion. Six optimisation formulations were analysed for stability by the Turbiscan stability analyser. Initial mean backscattering data indicated that the formulation containing the highest concentration BGNSF and lowest concentration oil would yield the most stable emulsion; which was further confirmed by the backscattering profiles. Furthermore backscattering kinetics clearly showed that BGNSF concentration determined the stability of each emulsion. The optimal orange oil-in-water beverage emulsion was thus identified as the emulsion containing 30% brown BGNSF and 6% orange oil.

The following conclusions can therefore be drawn from this study:

1. Insoluble and soluble dietary fibre fractions can be extracted from BGN varieties.
2. The method used for dietary fibre extraction was successfully applied. It is however noted that the method was costly, and future work will be focussed on alternative cost-effective extraction methods.
3. BGNIF's and BGNSF's have beneficial physicochemical and functional properties which are comparable or in some instances superior to commercial legume and other fibres.
4. BGNIF's can successfully be incorporated into white bread formulation, to provide added technological and nutritional benefits.
5. BGNIF enriched breads were acceptable to consumers.
6. BGNSF from brown variety stabilised an orange beverage emulsion, which indicates the potential of BGNSF as new polysaccharide stabiliser for use in the food industry.
7. South Africa Complete Patent (2014/04371) titled "Dietary fibre supplement" resulted from this study and was filed on 13 June 2014 (Appendix A).
8. Literature information from this study was contributed to a book chapter titled "Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*" (Appendix B).
9. Research outputs from this study were presented at several conferences (Appendix C).

APPENDICES

Appendix A: South Africa complete patent (2014/04371) application titled “Dietary Fibre Supplement”

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19 June 2014

Attention: Prof. Gary Atkinson
By e-mail: AtkinsonHopeG@cput.ac.za
CC: JideaniV@cput.ac.za; MartinK@cput.ac.za; RabiuH@cput.ac.za and diedericksf@gmail.com

Dear Gary

SOUTH AFRICA – COMPLETE PATENT APPLICATION NO. 2014/04371 IN THE NAME OF CAPE PENINSULA UNIVERSITY OF TECHNOLOGY ENTITLED DIETARY FIBRE SUPPLEMENT

Thank you for your instructions to file this complete South African patent application.

I confirm that the application was filed at the South African Patent Office on **13 June 2014**. A copy of the complete specification and drawings as filed and the electronic filing receipt is attached for your records.

You have been provided with copies of the Form P3 Declaration and Power of Attorney and the Form P26 statement on indigenous biological resources and traditional knowledge for signing. Please return the original signed documents for lodgement at the Patent Office at your earliest convenience. Note that these documents must be filed at the patent office before the acceptance date of the complete patent application.

Note that the South African Patent Office does not conduct a substantive examination therefore, as long as the formal requirements have been met, your patent application will be accepted and granted with the original specification. It is possible to apply for voluntary amendment after grant, but the application formalities are more onerous, and you may only amend the claims to narrow their scope, whereas prior to grant the scope of allowed amendment is broader. It is therefore recommended that if any voluntary amendments are necessary or desired, these are made as soon as possible, and certainly before grant.

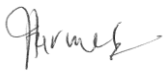
The acceptance date, before which all formal requirements must have been complied with, for this complete South African patent application is likely to be within about 10 months from the filing date. However, if desired, we can apply to extend acceptance for up to 18 months automatically. For example, this time may be used to get the formal documents required for the application in order, or to consider the outcome of examination in corresponding foreign applications and make pre-grant amendments.

Please let us know if you would like to extend acceptance at this stage, and if so, for what period.

Note that after the 18 month period, acceptance can be further extended for a three month period on application and payment of extension fees. Then a final extension of no prescribed period is allowable by the Registrar on "good cause shown" and payment of additional extension fees. We will remind you of these options if and when they become necessary.

Our invoice in connection with the filing of this application will follow via our e-billing system.

Yours sincerely



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Encl.

Appendix B: Book chapter titled "Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*

Chapter 8

Nutritional, Therapeutic, and Prophylactic Properties of *Vigna subterranea* and *Moringa oleifera*

Victoria Adaora Jideani and
Claudine Florett Diedericks

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/57338

1. Introduction

1.1. Bambara groundnut

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) [BGN] is an easy-to-cultivate legume seed classified under the family Fabaceae, sub-family Faboidea and genus *Vigna* [1]. Two botanical varieties exist, namely *V. subterranea* var. *spontanea* (wild varieties) and *V. subterranea* var. *subterranea* (cultivated varieties). BGN originated in West Africa from the Bambara district near Timbuktu and is now widely grown throughout tropical Africa, Indonesia, Malaysia, Sri Lanka, Central and South America and some parts of Northern Australia [2-4]. BGN is known by many common names such as Madagascar groundnut, baffin pea, voandzou, indhlubu, underground bean, nzama [Malawi], Epa-Roro [Nigeria], jugo beans [South Africa] and Nyimo beans [Zimbabwe] [1, 5]. Considered as one of the main attributes of BGN, is its tolerance of poor soils and drought, as well as its ability to yield in conditions in which groundnut fails completely. BGN also has an extremely tough seed coat, which makes it resistant to weevil attack and allows for storage of the seeds for long periods without loss [6]. Favourable characteristics making BGN an ideal crop includes its ability to be intercropped with other crops (i.e. maize, babala and sorghum), therefore not taking up areas designated to crops seen as more lucrative/important, its abundance in nitrogen which improves soil fertility and makes it useful in crop rotation, and the possibility to be grown without the use of expensive chemicals and fertilisers which are usually difficult to obtain in isolated areas [4].

BGN is propagated by its seeds which can be bought at local markets or are retained from the previous harvest. The larger seeds are used for cultivation and to retain maximum viability the seeds are dehulled before sowing [5]. As a leguminous annual short-day plant, BGN is

grown for its underground seeds. Similar to the peanut, the BGN plant grows close to the ground and pods and seeds are formed on or below the soil surface [5]. Depending on the cultivar and weather conditions, the BGN plant matures in three to six months. The flowers and pods have been identified as essential parts of the plant [7]. The onset of flowering is 30 – 35 days after sowing, followed by pod development 30 days after the fertilisation process and the seeds developing after the pods in ten days. Pods are approximately 1.5 cm long, they may be wrinkled and slightly oval or round shaped containing one to two seeds. Pod colour varies from yellowish-white for unripe pods to yellowish-brown or purple for mature pods [2, 6]. BGN seeds are usually round, hard and smooth and vary in size. The colour of the seeds vary from black, dark-brown, red, white, cream or a combination of these colours and it may also be speckled with or without hilum colouration [1,6]. Illustrations of the BGN plant and various seed varieties are shown in Figure 1.

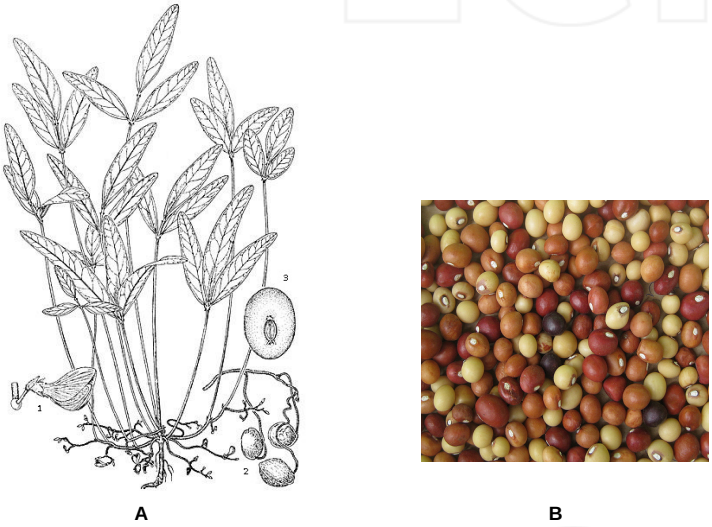

Figure 1. Bambara groundnut plant and seeds (A) Bambara groundnut flowering plant (1 – flower, 2 – fruits, 3 – seed); (B) Several varieties of Bambara groundnut seeds [8-9]

1.2. *Moringa oleifera*

The family of plants Moringaceae consists of 13 species outlined in Table 1. Out of the 13 species only *M. oleifera* has been accorded extensive research and development. They are important multipurpose crops in Africa and India. The species reported to have originated in India and Africa, are now grown around the world. Major production include Ghana, Senegal and Malawi, smaller production are in New Zealand and Fiji and more recent production in Nicaragua and Bolivia [10]. *Moringa* species are highly tolerant to arid conditions due to the formation of very large tuberous roots, and hence are often important famine foods [11]. Some common names for *M. oleifera* are detailed in Table 2. *Moringa* is a medium sized tree of 10 m

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height, with a straight trunk (10-30 cm thick), whitish or gray, corky bark with longitudinal cracks. It has a tuberous taproot whose presence helps the species' tolerance to drought conditions. The tree is normally umbrella shaped with a lax crown of graceful, airy foliage, whose feathery effect is due to the finely tripinnnate division of the leaves (Figure 2). The leaves are densely crowded at the tops of the branchlets [12].

Species	Origin
Moringa oleifera	India
M. drouhardii	Madagascar
M. cocanensis	India
M. arborea	North Eastern Kenya
M. hildebrandtii	Madagascar
M. oleifera	India
M. borziana	Kenya and Somalia
M. ovalifolia	Namibia and extreme southwestern Angola
M. peregrina	Horn of Africa, Red sea, Arabia
M. longituba	Kenya, Ethiopia, Somalia
M. stenopetala	Kenya, Ethiopia
M. pygmaea	Northern Somalia
M. rivae	Kenya, Ethiopia
M. ruspoliana	Kenya

[1]Adapted from [12]

Table 1. *Moringa* species[1]

2. Food uses of the crops

2.1. Bambara groundnut

Primarily grown for human consumption, BGN seeds are consumed in various ways in both immature and fully matured form. Whilst immature, the BGN seeds may be consumed fresh (raw), grilled or it may be boiled before consumption. These seeds are also more palatable compared to the mature seeds which are hard. To soften the mature seeds and render them more pleasant tasting and sweet, the seeds are boiled or roasted [7, 5]. Immature seeds are frequently consumed as a snack by boiling the fresh seed with salt or roasting the seeds, and may also be pounded with or without hulls and boiled into a stiff porridge [8, 4, 13]. Mature seeds may be consumed as is by boiling in water, or it is often ground into flour and consumed as porridge by mixing the flour with butter or oil. The seeds may also be dried, boiled and

consumed with plantains or maize [7-8]. Traditional uses of BGN inherent to certain areas in Africa are summarised in Table 3. Despite the many uses of BGN, the crop still remains underutilised due to several negative connotations such as being traditionally grown by women, an indigenous crop consumed by the poor in rural areas (from there the name "a poor man's food"), not being considered a lucrative cash crop and the difficulty in cooking and costs (including time, water and fuel) associated with cooking the seeds [4].

Language	Common name
English	*Moringa*, horseradish tree, drumstick tree, sujuna, ben tree, ben oil tree
French	Ben ailé, ben oléifère, benzolive, arbre radis du cheval
Spanish	Ben, árbol del ben, paraiso, morango, *Moringa*
Portuguese	acácia branca, marungo, muringa, moringuiero; cedro (Brazil)
Arabic	ruwag, alim, halim, shagara al ruwag (Sudan)
Swahili	mzunze, mlonge, mjungu moto, mboga chungu, shingo
German	Behenbaum, Behenussbaum, flügelsaniger Bennussbaum, Pferderettichbaum
Italian	Sàndalo ceruleo Fon: kpatima, yovokpatin,kpano,yovotin
Gun	èkwè kpatin, kpajima
Nigeria	
Yoruba & Nago	èwè igbale, èwè ile, èwè oyibo, agun oyibo, ayun manyieninu, ayèrè oyibo
Fulani	gawara, konamarade, rini maka, habiwal hausa
Hausa	zogall, zogalla-gandi, bagaruwar maka, bagaruwar masar, shipka hali, shuka halinka, barambo, koraukin zaila, rimin turawa
Ibo	Ikwe oyibo
Senegal	nebeday
Philippines	malunggay or malungai (Tagalog)
India	sujuna, sajina, lopa, horseradish or drumstick tree
Haiti	benzolive (Haitian Creole)

[1]Source: [12]

Table 2. *Moringa* common names[1]

Several research investigations are therefore aimed at highlighting the potential value of BGN as a sustainable food security crop. As in [14], milk was prepared from BGN by soaking the seeds in water, followed by homogenisation of the liquid and removal of the insoluble material. Acceptable BGN milk was obtained, and sensory analysis revealed panellists' preference for BGN milk in colour and taste compared to milk produced from soybean, cowpea and pigeon-

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pea. More recently, the functional properties of BGN flour and protein and starch fractions have also been investigated, as a means of better utilisation of this underutilised crop in food applications [15-19, 3].

2.2. *Moringa oleifera*

Moringa tree yields at least four different edibles namely pods, leaves, seeds and roots [12]. Figure 3 outlines some of the food uses of *Moringa*. The immature pods are the most valued and widely used of all the tree parts as it contains all the essential amino acids along with many vitamins and other nutrients. The tender pods have the general characteristics of a succulent string bean. It can be eaten raw or prepared like green peas or green beans. In India, they are usually added to curries and sometimes sliced, blanched and canned. The mature pods quickly turns tough as thick as a pencil and are too fibrous to eat like the string beans. In that form they are called drumsticks. However, they are cut into pieces to release the sweet frothy inside material which are well known ingredients in pickles in India.

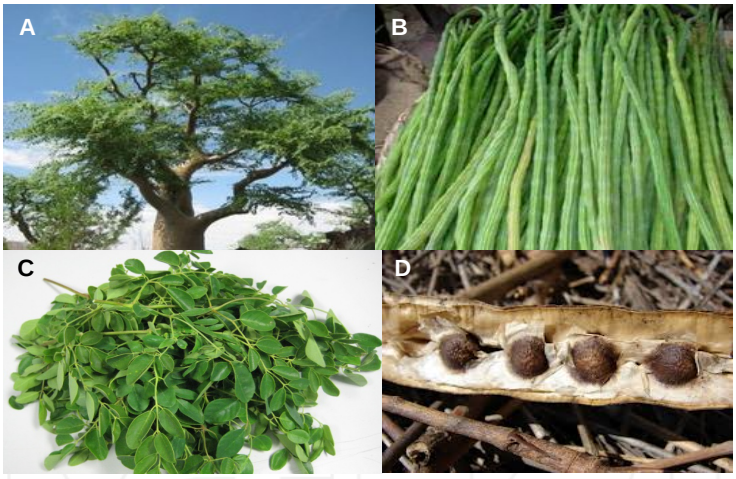

Figure 2. Part of *Moringa* tree (A) *Moringa* tree; (B) *Moringa* pods; (C) *Moringa* leaves and (D) *Moringa* pods with the seeds (www.ilove*Moringa*.com)

The fresh leaves are eaten as greens, in salads, in vegetable curries, as pickles and for seasoning. The dried leaves are crushed or pound and sifted into leaf powder which can then be added to sauces and foods as condiment. The flowers must be cooked and are eaten either mixed with other foods or fried in batter, and have been shown to be rich in potassium and calcium.

The seeds are often referred to as peas and can be used from the time they appear until they turn yellow and their shells begin to harden. They can be cooked like green peas. Hardened mature seeds are bitter and can be pressed yielding 38 – 40% of non-drying, edible oil which is clear, sweet and odourless and never becomes rancid and burns without smoke; its nutri-

tional value resembles olive oil [20]. The seed powder can be used for water treatment where the powder coagulates solids and removes 90 – 99% bacteria.

The thickened root is used as substitute for horseradish although this is now discouraged as it contains alkaloids, especially moriginine, and a bacteriocide, spirochin, both of which can prove fatal following ingestion. Older roots and root bark are good sources of tanning agents.

Country	BGN food uses	Source
Cameroon	Testa-free fresh seeds – consumed as a complete meal by cooking with seasoning, or ground to prepare a traditional pudding sometimes with addition of taro leaves	[21]
Northern Ghana	Dry BGN seeds – boiled and crushed seeds used to form cakes/ balls followed by frying and adding to stews; BGN is also made into a paste and used in traditional dishes 'tubani' (steamed bean paste) and 'koose'/'akla' (fried bean paste)	[22-23]
Southern Ghana	'Aboboi' – prepared by soaking BGN seeds overnight followed by boiling (with/without capsicum pepper and salt) to produce a type of porridge/blancmange; served with 'gari' or plantain (ripe, fried or mashed)	
Kenya – Kambe & Giriama tribes	Dry BGN seeds are prepared by removal of the seed coat through pounding, winnowing and boiling the seeds until cooked; cooked seeds are pounded and mixed with coconut juice – this preparation is cooked and stirred until smooth, and served with 'ugali' or rice	[24]
Nigeria	Paste prepared from BGN flour used in preparation of 'moi moi' and 'akara' (bean balls); 'okpa' (steamed gel prepared by slurry of BGN)	[25-26]
South Africa	BGN (sometimes with peanuts) are added to millet or maize and the mixture boiled to form a stiff dough; this dough is salted and made into a ball known as 'tshidzimba' (Venda), 'sekome' (Sesotho) or 'tihove' (Shangaan)	[27]

Table 3. Some food uses of Bambara groundnut in parts of Africa

3. Nutritional characteristics

3.1. Bambara groundnut

BGN seeds contain on average 63% carbohydrate, 19% protein and 6.5% fat; amounts which are regarded as sufficient to make the seed a complete food [1]. Reference [4] compared the nutritional composition of BGN with more commonly utilised and commercialised grain

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legumes, and BGN compared favourably (see Table 4). The high carbohydrate content of BGN is mainly composed of starch and non-starch polysaccharides [1], fractions which are important in the human diet providing energy and imparting several physiological functions. BGN is also rich in calcium, potassium, iron and nitrogen [4, 6]. In [28] the proximate composition of seeds, flour and seed coats from different BGN varieties were compared. Results for BGN seeds and flour showed no big differences, concluding that the inherent nutrients would be provided in either raw or processed (milled) form. Nti [22] evaluated the chemical composition of five BGN varieties as well as the effects of different processing conditions on the chemical, mineral and anti-nutritional composition of BGN flour samples. The moisture content of all varieties (ranging from 8.8 ± 0.22 – 9.8 ± 0.23%) indicated good storage stability of BGN seeds. An increase in tannins content were observed in darker-coloured varieties, with black white-eye BGN having the highest tannin content (14.92 ± 0.85 mg CE/g).

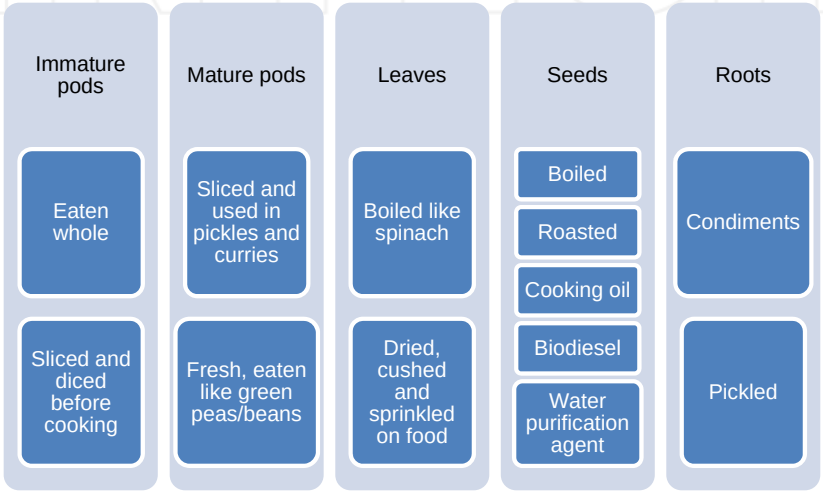

Figure 3. Some food uses of *Moringa* tree

These anti-nutritional components are mainly found in the seed coat and as in common beans, their concentration are correlated with the colour of the seeds [1]. Dehulling and boiling with dehulling having significant effects on the protein and tannins content of all varieties. Protein content which was highest in the undehulled (27.35 ± 0.27%) black white-eye variety as compared to the other varieties, increased significantly [$p < 0.05$] when dehulled (28.55 ± 0.26%) and boiled with dehulling (28.61 ± 0.51%). Tannins content in undehulled black white-eye BGN flour (15.40 ± 0.39 mg CE/g) decreased significantly [$p < 0.05$] when the sample was dehulled (1.16 ± 0.12 mg CE/g) and even more so when boiling and dehulling (0.09 ± 0.02 mg CE/g). These results demonstrate the positive effect of processing conditions on the nutritional properties of BGN, which could lead to increased utilisation in especially weaning products in which high-protein formulations are important.

The highly nutritious content of BGN and its unusually high content of the sulphur-containing essential amino acid methionine, makes BGN an important crop to consider for food security [4].

	Bambara groundnut	Soybean	Chickpea	Cowpea
Calories (kCal)	390.0	416.0	364.0	343.0
Protein (g)	20.8	36.5	19.3	23.8
Carbohydrates (g)	61.9	30.2	60.6	59.6
Fat (g)	6.6	19.9	6.0	2.1

[1] Adapted from [4]

Table 4. Nutritional composition of BGN and some commonly utilised legumes[1]

3.2. *Moringa oleifera*

M. oleifera leaves are good source of protein, β-carotene, vitamins, A, B, C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals and various phenolic compounds [29-30]. *Moringa oleifera* leaf powder (25 g daily) is said to give a child the recommended daily allowance for protein (42%), calcium (125%), magnesium (61%), potassium (41%), iron (71%), vitamin A (272%), and vitamin C (22%). Gram for gram, *M. oleifera* leaves contain seven times the vitamin C in oranges, four times the calcium in milk, four times the β-carotene in carrots, twice the protein in milk and three times the potassium in bananas [31-33].

Leaves of *M. oleifera* are rich in palmitic (16:0) and linolenic (18:3) acids whereas the seeds are predominated by oleic acid (18:1). The roots are rich in palmitic and oleic acid whereas the stems and twigs are rich in palmitic acid [34]. It is becoming popular not only among the lower socio-economic class, but in the entire society irrespective of one's socio-economic background and health status.

4. Phytochemical properties of the crops

4.1. Bambara groundnut

Some phytochemistry studies have been done on species from the genus *Vigna*, with most focussing on *V. unguiculata* (cowpea) and limited information available on *V. subterreanea*. Pale et al. [35] investigated the anthocyanins present in bambara groundnut through column and preparative thin-layer chromatography. Three anthocyanins (delphinidin 3-*O*-β-glucoside, petunidin 3-*O*-β-glucoside and malvidin 3-*O*-β-glucoside) were identified. Anthocyanins have many beneficial effects on health, and further investigation into the health properties associated with BGN consumption is needed. In a study by [36], eleven species of *Vigna* were surveyed for canavanine, proanthocyanidin and flavonoid profiles. Canavanine, delphinidin

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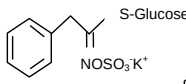
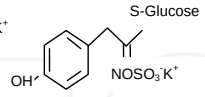
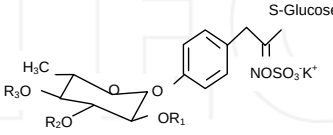
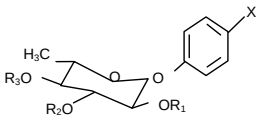
and cyanidin were absent in BGN seeds. The absence of canavanine is consistent in the species of *Vigna*. The flavonoid profiles revealed that the four BGN varieties studied accumulated four types of kaempferol glycosides. In all *Vigna* species, the prevalent flavonoid appears to be kaempferol. Kaempferol-3-*O*-glucoside-7-rhamnoside seemed to be restricted to BGN. As a polyphenol antioxidant, kaempferol imparts many health benefits and reduces the risk of many chronic illnesses such as cancer [37]. A recently published article by [38] also reveals the possible components in BGN which could have beneficial effects on health in their study on the effects of gas flaring on the African breadfruit and BGN. Valuable information on the phytochemical properties of BGN was found with high concentrations in the unpolluted samples for oxalate (0.38 ± 0.04%), saponin (0.24 ± 0.02%); vitamin E (3.18 ± 0.15 mg/100 g), vitamin C (1.17 ± 0.20 mg/100 g), vitamin A (26.05 ± 0.14 mg/100 g) and niacin (2.10 ± 0.06 mg/100 g). The concentrations of oxalate, saponin, alkaloid and flavonoid were increased by gas flaring, whilst the concentrations of vitamins were significantly [$p < 0.05$] reduced. Vitamin A which is important for maintaining good eye-sight and preventing eye diseases [39], were significantly higher [$p < 0.05$] in the BGN seeds as compared to the other vitamins detected. The information available on phytochemical components of BGN seeds is promising, and should be further investigated to determine and highlight their specific effects on human health, which could greatly influence the current underutilised status of this crop.

4.2. *Moringa oleifera*

Strickly speaking, phytochemicals are non-nutritive chemicals produced by plants which may have an impact on health, or on flavour, texture, smell or colour of the plants. Plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans against diseases. The phytochemicals include the alkaloids, anthocyanins, carotenoids, coumestans, flavan-3-ols, flavonoids, hydroxycinnamic acids, isoflavones, lignans, monophenols, monoterpenes, organosulfides, phenolic acids, phytosterols and saponins. Each phytochemicals work differently. *M. oleifera* contains various phytochemicals namely, carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics [40, 29]. Table 5 details the phytochemicals found in *M. oleifera*. *Moringa* species are rich sources of various phytochemicals including uncommon sugar-modified glucosinolates, although there are only details on quantity and profiles for *M. oleifera*, *M. peregrine* and *M. stenopetala* [34, 41-42]. The predominant glucosinolate is 4-O-(α-L-rhamnopyranosyloxy)-benzylglucosinolate (glucomoringin) and depending on the tissues three mono-acetyl-rhamnose isomers of this glucosinolate have also been detected [41, 43]. Chlorogenic acids and flavonols have been reported in different tissues of *M. oleifera* and *M. stenopetala* but there is no information for other *Moringa* species [34, 40-41, 44-45]. The flavonoid profile was found to be quite complex and was predominated by flavonol glycosides (glucosides, rutinosides and malonylglucosides of quercetin, kaempferol and isorhamnetin). The predominant core aglycones are flavonols: quercetin > kaempferol > isorhamnetin. The leaves had the highest and most complex flavonoid contents, and no flavanoids were detected in roots or seeds. The antioxidant activity of leaves from *M. oleifera* was shown to be very high due to the high concentrations of polyphenolics [46-47]. Therefore *M. oleifera* tissues could be an important dietary source of antioxidant polyphenolics.

Guevara et al. [48] isolated eight compounds from the seeds of *M. oleifera* namely, O-ethyl-4-(α-L-rhamnosyloxy)-benzyl carbamate, 4-(α-L-rhamnosyloxy-benzyle isothiocyanate, niazimicin, niazirin, β-stiosterol, glycerol-1-(9-octadecanoate), 3-O-(6′-O-oleoyl-β-d-glucopyranosyl)-βsitosterol and β-sitosterol-3-O-β-d-glucopyranoside. 4-(α-L-rhamnosyloxy-benzyle isothiocyanate, niazimicin and β-sitosterol-3-O-β-d-glucopyranoside showed significant inhibitory activity against Epstein-Barr virus-early antigen (EBV-EA) and niazimicin in particular was found to have potent antitumor promoting acitivity in vivo in the two-stage carcinogenesis in mouse skin. They proposed that niazimicin could be a potent chemo-preventive agent in chemical carcinogenesis. Beta-sitosterol acts against some form of cancer and was found to reduce the growth of prostate and colon cancer cells. Other medical benefits of beta-sitosterol are boosting of immune defense, anti-inflammatory, normalising blood sugar, healing of ulcers and alleviating cramps.

Niaziridin and niazirin are present in leave and pods, respectively and are not detected in the bark of *M. oleifera*. Relatively higher amount of niazirin is present in leaves in comparison to the pods, while niaziridin content was about three times higher in the pods than the leaves [49]. Niaziridin rich fraction of *M. oleifera* pods enhances the bioactivity of commonly used antibiotics such as rifampicin, tetracycline and ampicillin against gram positive and negative bacteria and also facilitates the absorption of drugs, vitamins and nutrients through the gastro-intestinal membrane thus increasing their bio-availability [50]. Therefore, niaziridin can be used in combination therapy with drugs and nutrients resulting in reduced drug associated toxicity, reduced cost and duration of chemotherapy [49]. Hence, fruits of *M. oleifera* contain antitumor and anti-inflammatory compounds of the glycoside type (i.e. niazirin, niazimicin, niazicin A).

Phytochemical	Structure	Location	Ref.
Glucosinolates			
Benzylglucosinolate (Glucotropaeolin)			
4-Hydroxybenzylglucosinolate (Sinalbin)			
4-O-(α-L-Rhamnopyranosyloxy)-benzylglucosinolate (Glucomoringin) (G2) (R1, R2, R3 = H)		All tissues except the roots	
4-O-(α-L-Acetyl-rhamnopyranosyloxy)-benzylglucosinolate (G3-G5) (R1 & R2 = H, R3 = Ac; R1 & R3 = H, R2 = Ac; R1 = Ac; R2 & R3 = H)		Roots	
Hydrolysis Products & Related Derivatives			
4-O-(α-L-Rhamnopyranosyloxy)-benzylisothiocyanate (R1 = R2 = R3 = H, X = N = C = S) Niazirin (R1 = R2 = R3 = H, X = CN)		Leaves and pods Roasted seeds	[48]

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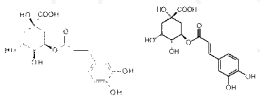
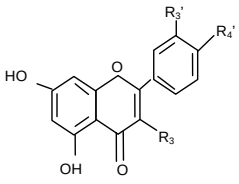
Phytochemical	Structure	Location	Ref.
Niazirinin (R1 = R2 = H, R3 = Ac, X = CN Niazimin A/B (R1 = R2 = H, R3 = Ac, X = CH2-NH-CO-OEt) Niazinin A/B (R1 = R2 = R3 = H, X = CH2-NH-(C=S)-OMe) Niazicin A/B (R1 = R2 = H, R3 = Ac, X = CH2-NH-(C=S)-OMe) Niazimicin (R1 = R2 = R3 = H, X = CH2-NH-(C=S)-OEt) Niaziminin A/B (R1 = R2 = H, R3 = Ac, X = CH2-NH-(C=S)-OEt)			
Phenolics			
3-Caffeoylquinic acid (3-CQA) (Neochlorogenic acid) 5-Caffeoylquinic acid (50CQA) (Chlorogenic acid)		All tissues except the roots, pods and seeds	[41]
Major flavonoids (K = Kaempferol, Q = Quercetin) K 3-O-Rutinoside ((R3 =-GlcRha, R3′ = H & R4′ = OH) (F7) K 3-O-Glucoside (R3 = -Glc, R3′ = H, R4′ = OH) (F9) K 3-O-(6″-Malonylglucoside) (R3 = -GlcMalm R3′ = H, R4′ = OH) (F13) Q 3-O-Rutinoside (R3 = -GlcRha, R3′ & R4′ = OH) (F4) Q 3-O-Glucoside (R3 = -Glc, R3′ & R4′ = OH) (F6) Q 3-O-(6″-Malonylglucoside) (R3 = -GlcMal, R3′ & R4′ = OH) (F8)			

[1]Source: [34]

Table 5. Phytochemicals found in *M. oleifera*[1]

Polyphenolic compounds exist widely in the plant kingdom and are used in humans to modulate lipid peroxidation involved in atherogenesis, thrombosis and carinogenesis due to their antioxidant activity and anti-inflammatory action [40, 51]. Both aqueous and acetone extracts of *M. oleifera* leaves have potent antioxidant activities; however, Moyo et al. [52] reported higher values of phenols, flavonoids, flavonol and proanthocyanidins in acetone extract of *M. oleifera* leaves than the aqueous extract. Similar observation was reported by other researchers [40, 46, 53-54]. The ability of the extracts to adsorb and neutralise free radicals or decompose peroxides are attributed to the synergistic effect of phenolic compounds in the *M. oleifera*. The redox properties, presence of conjugated ring struc-

tures and carboxylic group which can inhibit lipid peroxidation are responsible for its ability as free radical scavengers [55].

The aqueous extract of leaf (LE), fruit (FE) and seed (SE) of *M. oleifera* could significantly inhibit the OH-dependent damage of pUC18 plasmid DNA with an activity sequence of LE > FE > SE. Gallic acid, chlorogenic acid, ellagic acid, ferulic acid, kaempferol, quercetin and vanillin were present in the extracts. The leaf extract was comparatively higher in total phenolics [105.04 mg gallic acid equivalents (GAE/g)], total flavonoids [31.28 mg quercetin equivalents (QE/g)] and ascorbic acid (106.95 mg/100 g) with better antioxidant activity (85.8%), anti-radical power (74.3), reducing power [1.1 ascorbic acid equivalents (ASE/ml)], inhibition of lipid peroxidation, protein oxidation, OH-induced deoxyribose degradation and scavenging power of superoxide anion and nitric oxide radicals than did the FE, SE and standard α-tocopherol [56]. Many gram negative bacteria such as *Erwinia carotovora*, *Enterobacter agglomerans*, *Chromobacterium violaceum* and *Pseudomonas aeruginosa* use N-acyl homoserine lactones (AHLs) signal molecules to monitor their own population density. At a threshold population density, AHLs interact with cellular receptors and trigger the expression of a set of target genes including virulence, antibiotic production, biofilm formation, bioluminescence, mobility and warming, in a process called "quorum sensing" (QS) [57]. The discovery of the QS system and its critical role in bacteria virulence and survival has revealed a novel way to attack and attenuate bacterial pathogenicity. The major advantage of this novel strategy for anti-infective therapy is that it circumvents the problem of antibiotic resistance, which is intimately connected to the use of conventional antibacterial agents, as it specifically interferes with the expression of pathogenic traits rather than to impede growth of the bacteria. The efficacy and toxicity of previous reported QS blockers (halogenated furanones) have been important concerns. Hence, attention has been focused on identification of such QS blockers from natural and non-toxic sources for the development of novel non-antibiotic drugs for treating bacterial diseases in humans as well as in other animals. Singh et al. [56] reported that the leaf and the fruit extracts of *M. oleifera* inhibited violacein production, a QS-regulated behaviour in *Chromobacterium violaceum* 12472. This provides evidence on *M. oleifera* as natural antioxidant for its capacity to protect organism and cell from oxidative DNA associated with aging, cancer and denegerative diseases as well as inhibit lipid peroxidation and bacterial QS. Thus, *M. oleifera* may serve as an ideal ingredient for functional food, nutraceutical and bio-pharmaceutical industries.

The seeds of *Moringa oleifera* contain 4 (α-L-Rhamnosyloxy) benzyl isothiocyanate and benzyl isothiocyanate. These are antimicrobial agents effective against several bacteria and fungi. The minimal bactericidal concentration in vitro is 40 μmol/l for *Mycobacterium phlei* and 56 μmol/l for *Bacillus subtilis* [58]. Singh et al. [10] identified ten phenolic compounds (gallic acid, p-coumaric acid, ferulic acid, caffeic acid, protocatechuric acid, cinnamic acid, catechin, epicatechin, vanillin and quercetin) from defatted *M. oleifera* seed flour. These natural plant phenolics could be a good source of antioxidants and antimicrobials for food and pharmaceutical industries.

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5. Therapeutic and prophylactic properties of the crops

5.1. Bambara groundnut

The medicinal role of BGN is mainly based on information obtained from communities in several parts of Africa, where this crop is reportedly responsible and useful for treatment of various ailments. As a treatment for diarrhoea, a mixture of BGN and water from boiled maize are consumed. Raw BGN seeds are chewed and swallowed by pregnant women to alleviate the nausea associated with pregnancy [7]. The medicinal value of the crop have also been highlighted and reviewed by [59]. The following uses of BGN as traditional medicine have been noted by the authors (i) In several countries in sub-Saharan Africa, BGN plays an important role in the diets of especially young rural children as it helps in overcoming the protein deficiency Kwashiorkor; (ii) The Igbos tribe in Nigeria use the seeds for treatment of venereal diseases; (iii) To treat polymenorrhea it is recommended that BGN seeds be roasted before consumption; (iv) The water in which BGN seeds are boiled is used as treatment for internal bruising, and a mixture of water and crushed seeds are prescribed for treatment of cataracts; (v) BGN seeds have the highest concentration of soluble fibre as compared to other beans; this could contribute to the reduction of heart disease incidence and prevention of colon cancer; (vi) Surveys amongst local communities in northern Côte d'Ivoire revealed that the BGN seeds are mainly used for medical treatments as opposed to other parts of the plant. The seeds are used to treat anemia, ulcers (black BGN variety mixed with an unidentified plant) and menorrhagia during pregnancy (hemostatic drink prepared by a mixture of BGN flour and *Pupalia lappacea* (L.) Amaranthaceae dissolved in water). The traditional uses of BGN to treat several ailments are noteworthy, and present a gap for detailed study on the pharmaceutical value of the crop. This would provide yet another means of highlighting the potential of BGN as an underutilised legume and tap into ways of encouraging more sustained production and use of BGN.

5.2. *Moringa oleifera*

Besides the rich nutritional value of *M. oleifera* it has curative and prophylactic properties [24]. Almost all the parts (root, bark, gum, leaf, pods, flowers, seed and seed oil) of M. oleifera have been used for various ailments including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, haematological, hepatorenal disorders, diabetes mellitus, CNS depressant, and for antifertility effect [40]. The plant has been used for the treatment of ascites, rheumatism and for the enhancement of cardiac function. The seed extract have been reported to be administered nasally in diseases like rhinitis and the dried seeds used successfully as an 'anti-allergic' agent by the Ayurvedic practitioners [60]. Mahajan [61] reported an antiarthritic activity of ethanolic extract of seeds of *M. oleifera* against chemical induced rheumatoid arthritis as well as an antiasthmatic activity against immune-mediated inflammatory responses in rat [62]. *M. oleifera* seed extract can act against CCl4-induced liver injury and fibrosis in rats by a mechanism related to its

antioxidant properties, anti-inflammatory effect and its ability to attenuate the hepatic stellate cells activation [63].

Siddhuraju and others [40] reported that leaf extracts (water, aqueous methanol, aqueous ethanol) were capable of scavenging peroxyl and superoxyl radicals. The major bioactive compounds of phenolics were found to be flavonoid groups such as quercetin and kaempferol. *Moringa* leaves are therefore potential source of natural antioxidants. The ethanol leaf extract of *Moringa oleifera* is used for hypertension [64-66]. The leaves are used as hypocholesterolemic and hypoglycemic agents [64, 67-68]. Additionally, the leaves have been reported for its antitumour [69], antioxidant [46, 54, 70], radio-protective [71-72], anti-inflammatory/diuretic properties [73], antihepatotoxic [74], antifertility [75], antiurolithiatic [76] and analgesic activities [77]. Choudhary and others [78] reported that ethanolic root-bark extract of *M. oleifera* possesses valuable antiulcer, antisecretory and cytoprotective activity in rats and thus can be used as source for an antiulcer drug.

An old report from Southeast Asia says a decoction of bark stimulates menses and is used for "morning after" birth control. In parts of West Africa, *Moringa* leaves or juice are taken for diabetes and high blood pressure [12]. Traditionally, leaves, fruits, roots and seeds of this plant are used for treating abdominal tumors, hysteria, scurvy, paralytic attacks, helminitic, bladder, prostate troubles, sores and skin infections [32].

Moringa oleifera possess genotoxicity at a high dose 3000 mg/kg b.wt of the powdered aqueous extract. However, intake is safe at levels ≤ 1000 mg/kg b.wt. [79].

6. Harnessing the rich nutritional and health properties of Bambara groundnut and *M. oleifera* for human nutrition

Bambara groundnut is a leguminous crop with great potential of sustaining the dietary needs of many people in both rural and urban communities. This indigenous African legume have been frowned upon as a 'poor man's food', but as more information emerges on the rich nutritional profile of BGN the importance of this crop to human nutrition is becoming more evident. In our laboratory we have demonstrated that Bambara groundnut could be used to produce a probiotic beverage as well as a rich source of soluble and insoluble fibre that can be used to enhance the nutrition and textural properties of white bread [80-81].

Moringa could be incorporated into programs on malnutrition. With four times the beta-carotene of carrot, *Moringa* has especial potential for programs dealing with avitaminosis, the vitamin A deficiency that causes 70 percent of childhood blindness. Consumption of diet supplemented with *M. oleifera* leaves could protect against diseases induced by oxidative stress. Many *Moringa* nutritional supplements exist in the market including *Moringa* dry leaf powder, capsules, nutrition shake and health booster. Perhaps using the multi mix approach of food product development more food products could be developed especially for programs on malnutrition.

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References

[1] Bamishaiye, O.M., Adegbola, J.A. and Bamishaiye, E.I. Bambara groundnut: an under-utilized nut in Africa. Advances in Agricultural Biotechnology 2011; 1 60-72.

[2] Swanevelder, C.J. Bambara—Food for Africa (*Vigna subterranea* – bambara groundnut). Pp. 1-16. South Africa: National Department of Agriculture; 1998. p1-16.

[3] Eltayeb, A.R.S.M., Ali, A.O., Abou-Arab, A.A. & Abu-Salem, F.M. Chemical composition and functional properties of flour and protein isolate extracted from bambara groundnut (*Vigna subterranea* (L.) Verdc.). African Journal of Food Science 2011; 5(2) 82-90.

[4] De Kock, C. Bambara groundnut. http://www.underutilized-species.org/documents/Publications/bambara_groundnut_paper.pdf (accessed 03 September 2013)

[5] Stephens, J.M. Bambara Groundnut—*Voandzeia subterranea* (L.) Thouars. Gainesville, Florida: University of Florida; 2012. http://edis.ifas.ufl.edu/mv014 (accessed 11 June 2013).

[6] Mkandawire, C.H. Review of bambara groundnut (*Vigna subterranea* (L.) Verdc.) production in Sub-Sahara Africa. Agricultural Journal 2007; 2(4) 464-470.

[7] Directorate Plant Production (DPP). Production guidelines for Bambara groundnuts. Pretoria, South Africa: Department of Agriculture, Forestry and Fisheries; 2011. p1-10.

[8] Brink, M., Ramolemana, G.M. and Sibuga, K.P. *Vigna subterranea* (L.) Verdc. In: *Plant Resources of Tropical Africa/ Ressources végétales de I'Afrique tropicale* (edited by M. Brink & G. Belay). Wageningen, Netherlands: PROTA4U; 2006. http://www.prota4u.org/search.asp. (accessed 5 September 2013).

[9] Rulkens, T. Bambara groundnut (*Vigna subterranea*) from Buzi district in Mozambique. http://www.flickr.com/photos. (accessed 5 September 2013).

[10] Singh, R.S.G, Negi, P.S. and Radha, C. Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of *Moringa oleifera* seed flour. Journal of Functional Foods 2013 (Article in Press).

[11] Sena, L.P., VanderJagt, D.J., Rivera, C., Tin, A.C., Muhamadu, I., Mahamadou, O., Millton, M., Pastuszyn, A. And Glew, R.H. Analysis of nutritional components of eight famine foods of the Republic of Niger. Plant Foods for Human Nutrition 1998; 52 17-30.

[12] National Research Council (2006). Lost crops of Africa: Volume II: Vegetables. http://www.nap.edu/catalog/11763.html. (accessed 15 October 2013).

[13] Uebersax, M.A. and Occeña, L.G. Legumes in the diet. In: *Encyclopedia of Food Sciences and Nutrition*, 2nd Edition (edited by B. Caballero, L. Trugo & P.M. Finglas). New York: Elsevier Science Ltd; 2003. p3520-3528.

[14] Brough, S.H., Azam-Ali, S.N. and Taylor, A.J. The potential of bambara groundnut (*Vigna subterranea*) in vegetable milk production and basic protein functionality systems. Food Chemistry 1993; 47 277-283.

[15] Adebowale, K.O., Afolabi, T.A. and Lawal, O.S. Isolation, chemical modification and physicochemical characterisation of bambarra groundnut (*Voandzeia subterranean*) starch and flour. Food Chemistry 2002; 78 305-311.

[16] Adebowale, K.O. and Lawal, O.S. Comparative study of the functional properties of bambarra groundnut (*Voandzeia subterranean*), jack bean (*Canavalia ensiformis*) and mucuna bean (*Mucuna pruriens*) flours. Food Research International 2004; 37 355-365.

[17] Lawal, O.S., Adebowale, K.O. and Adebowale, Y.A. Functional properties of native and chemically modified protein concentrates from bambara groundnut. Food Research International 2007; 40 1003-1011.

[18] Sirivongpaisal, P. Structure and functional properties of starch and flour from bambara groundnut. Songklanakarin Journal of Science and Technology 2008; 30(1) 51-56.

[19] Yusuf, A.A., Ayedun, H. and Sanni, L.O. Chemical composition and functional properties of raw and roasted Nigerian benniseed (*Sesamum indicum*) and bambara groundnut (*Vigna subterranean*). Food Chemistry 2008; 111 277-282.

[20] Tsaknis, J., Lalas, S., Gergis, V., Douroglou, V. and Spiliotis. Characterisation of *Moringa oleifera* variety Mbololo seed oil of Kenya. Journal of Agricultural and Food Chemistry 1999; 47 4495-4499.

[21] Nguy-Ntamag, F.C. Country Reports: Cameroon. Bambara groundnut *Vigna subterranea* (L.) Verdc. Promoting the conservation and use of underutilized and neglected crops 9. In: Heller J, Begemann F, Mushonga J. (eds.). Proceedings of the workshop on Conservation and Improvement of Bambara Groundnut *(Vigna subterranea (L.) Verdc.)*. November 1995. Harare, Zimbabwe; 1997.

http://dx.doi.org/10.5772/57338

[22] Nti, C.A. Effects of bambara groundnut (*Vigna subterranea*) variety and processing on the quality and consumer appeal for its products. International Journal of Food Science and Technology 2009; 44 2234-2242.

[23] Doku, E.V. Country Reports: Ghana. Bambara groundnut *Vigna subterranea* (L.) Verdc. Promoting the conservation and use of underutilized and neglected crops 9. In: Heller J, Begemann F, Mushonga, J. (eds.). Proceedings of the workshop on Conservation and Improvement of Bambara Groundnut *(Vigna subterranea (L.) Verdc.)*. November 1995. Harare, Zimbabwe; 1997.

[24] Ngugi, G.W. Country Reports: Kenya. Bambara groundnut *Vigna subterranea* (L.) Verdc. Promoting the conservation and use of underutilized and neglected crops 9. In: Heller J, Begemann F, Mushonga J. (eds.). *Proceedings of the workshop on Conservation and Improvement of Bambara Groundnut (Vigna subterranea (L.) Verdc.)*. November 1995. Harare, Zimbabwe; 1997.

[25] Obizoba, I.C. Nutritive value of cowpea-bambara groundnut rice mixtures in adult rats. Nutrition Reports International 1983; 27 709-712.

[26] Uvere, P.O., Uwaegbute, A.C. and Adedji, E.M. Effects of malting on the milling performance and acceptability of bambara groundnut (*Voandzeia subterranea* Thouars) seeds and products. Plant Foods for Human Nutrition 1999; 54 49-57.

[27] Swanevelder, C.J. Country Reports: South Africa. Bambara groundnut *Vigna subterranea* (L.) Verdc. Promoting the conservation and use of underutilized and neglected crops 9. In: Heller J, Begemann F, Mushonga J. (eds.). *Proceedings of the workshop on Conservation and Improvement of Bambara Groundnut (Vigna subterranea (L.) Verdc.)* November 1995. Harare, Zimbabwe; 1997.

[28] Ojimelukwe, P.C. and Ayernor, G.S. Oligosaccharide composite and functional properties of flour and starch isolates from four cultivars of Bambara groundnut seeds. Journal of Food Science and Technology 1992; 29 319-321.

[29] Anwar, F., Latif, S., Ashraf, M. and Gilani, A.H. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytotherapy Research 2007; 21 17–25.

[30] Khalafalla, M.M., Abdellatef, E., Dafalla, H.M., Nassrallah, A.A., Aboul-Enein, K.M. and Lightfoot, D.A. Active principle from *Moringa oleifera* Lam. leaves effective against two leukemias and a hepatocarcinoma. African Journal of Biotechnology 2010; 9 8467–8471.

[31] Ramachandran, C., Peter, K.V. and Gopalakrishnan, P.K. Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. Economic Botany 1980; 34, 276–283.

[32] Fuglie, L.J. Isolation and structure elucidation of new nitrile and mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. Journal of Natural Products (1999a); 57 1256–1261.

[33] Fuglie, L.J. The Miracle Tree. *Moringa oleifera*: Natural Nutrition for the Tropics. Dakar: Church World Service; (1999b). p. 68.

[34] Amaglo, N.K.; Bennett, R.N.; Lo Curto, R.B., Rosa, E.A.S., Turco, V.L, Giuffrida, A.; Curto, A.L., Crea, F. and Timpo, G.M. Profiling selected phytochemicals and nutrients in different tissue of the multipurpose tree *Moringa oleifera* L., grown in Ghana. Food Chemistry 2010; 122 1047-1054.

[35] Pale, E., Nacro, M., Vanhaelen, M. and Vanhaelen-Fastré. Anthocyanins from Bambara Groundnut (*Vigna subterranea*). Journal of Agricultural and Food Chemistry 1997; 45 3359-3361.

[36] Onyilagha, J.C., Islam, S. and Ntamatungiro, S. Comparative phytochemistry of eleven species of *Vigna* (Fabaceae). Biochemical Systematics and Ecology 2009; 37 16-19.

[37] Chen, A.Y. & Chen, Y.C. A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. Food Chemistry 2013; 138(4) 2099-2107.

[38] Ujowundu, C.O., Nwagou, L.A., Ujowundu, F.N. & Belonwu, D.C. Effect of gas flaring on the phytochemical and nutritional composition of *Treculia Africana* and *Vigna subterranean*. British Biotechnology Journal 2013; 3(3) 293-304.

[39] Nwaogu, L.A. and Ujowundu, C.O. Effect of petroleum hydrocarbon pollution on the nutritional value of ripe guava (*Psidium guajava*) fruits grown in Imo State, Nigeria. International Journal of Biological and Chemical Sciences 2010; 4(2) 450-455.

[40] Siddhuraju, P. and Becker, K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. Journal of Agricultural and Food Chemistry 2003; 51(8) 2144-2155.

[41] Bennett, R.N., Mellon, F.A., Foidl, N., Pratt, J.H., DuPont, M.S., Perkins, L. and Kroon, P.A. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (Horseradish tree) and *Moringa stenopetala* L. Journal of Agricultural and Food Chemistry 2003; 51 3546–3553.

[42] Fahey, J.W., Zalcmann, A.T. and Talalay, P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 2001; 56 5–51.

[43] Kjaer, A., Malver, O., El-Menshawi, B. and Reischt, J. Isothiocyanates in myrosinase-treated seed extracts of *Moringa peregrina*. Phytochemistry 1979; 18 1485–1487.

[44] Lako, J., Trenerry, V.C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S. and Premier, R. Phytochemical flavonols, carotenoids and antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chemistry 2007; 101 1727–1741.

[45] Manguro, L.O.A. and Lemmen, P. Phenolics of *Moringa oleifera* leaves. Natural Products Research 2007, 21.

http://dx.doi.org/10.5772/57338

[46] Sreelatha, S. and Padma, P.R. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. Plant Foods for Human Nutrition 2009; 64 303–311.

[47] Verma, A.R., Vijayakumar, M., Mathela, C.S., & Rao, C.V. In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. Food and Chemical Toxicology 2009; 47 2196–2201.

[48] Guevara, A.P., Vargas, C., Sakurai, H. Fujiwara, Y., Hashimoto, K. Maoka, T., Kozuka, M., Ito, Y., Tokuda, H. and Nishino, H. An antitumor promoter from *Moringa oleifera* Lam. Mutation Research 1999; 440 181-188.

[49] Shanker, K, Gupta, M.M., Srivastava, S.K.; Bawankule, D.U.; Pal, A. and Khanuja, S.P.S. Determination of bioactive nitrile glycoside(s) in drumstick (*Moringa oleifera*) by reverse phase HPLC. Food Chemistry 2007; 105 376-382.

[50] Khanuja, S.P.S., Arya, J.S., Tiruppadiripuliyur, R.S.K., Saikia, D., Kaur, H., Singh, M., Gupta, S. C., Shasany, A.K., Darokar, M.P., Srivastava, S.K., Gupta, M.M., Verma, S.C. and Pal, A. Nitrile glycoside useful as a bioenhancer of drugs and nutrients, process of its isolation from *Moringa oleifera*. (United States Patent 2005; 6,858,588 B2).

[51] Frankel, E.N., & Meyer, A.S. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. Journal of the Science of Food and Agriculture 2000; 80 1925–1941.

[52] Moyo, B., Oyedemi, S., Masika, P.J. and Muchenje, V. Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. Meat Science 2012; 91, 441-447.

[53] Frum, Y., & Viljoen, A.M. In vitro 5-lipoxygenase and antioxidant activities of South African medicinal plants commonly used topically for skin diseases. Skin Pharmacology and Physiology 2006; 19 329–335.

[54] Atawodi, S.E., Atawodi, J.C., Idakwo, G.A., Pfundstein, B., Haubner, R., Wurtele, G., Bartsch, H. and Owen, R.W. Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of *Moringa oleifera* Lam. Journal of Medicinal Food 2010; 13 710–716.

[55] Oyedemi, S.O., Bradley, G. and Afolayan, A. J. In-vitro and in-vivo antioxidant activities of aqueous extract of Strychnos henningsii Gilg. African Journal of Pharmacy and Pharmacology 2010; 4 70–78.

[56] Singh, B.N., Singh, B.R., Singh, R.L., Prakash, D., Dhakarey, R., Upadhyay, G. and Singh, H.B. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. Food and Chemical Toxicology 2009; 47 1109-1116.

[57] Manefield, M., Rasmussen, T.B., Henzter, M., Steinberg, P., Kjelleberg, S. and Givsko, M. Halogenated furanones inhibit quorum sensing through accelerated LuxR turnover. Microbiology 2002; 148 1119–1127.

[58] Eilert, U., Wolters, B. and Nahrstedt, A. The antibiotic principle of seeds for *Moringa oleifera* and *Moringa stenopetala*. Planta Medica 1981; 42(5) 55-61.

[59] Koné. M., Paice, A.G. and Touré, Y. Bambara groundnut [*Vigna subterranea* (L.) Verdc. (Fabaceae)] usage in human health. In: *Nuts and Seeds in Health and Disease Prevention* (edited by V.R. Preedy, R.R. Watson & V.B. Patel). UK: Elsevier Inc.; 2011. p. 192-194.

[60] Chopra, R.N., Chopra, I.C., Handa, K.L., Kapur, I.D. (Eds.), 1938. Chopra's Indigenous Drugs of India. UN Dhur and Sons, Calcutta, India, 1938, p. 110.

[61] Mahajan, S.G., Mali, R.G. and Mehta, A.A. Protective effect of ethanolic extract of seeds of *Moringa oleifera* Lam. against inflammation associated with development of arthritis in rats. Journal of Immunotoxicology 2007a; 4 39–47.

[62] Mahajan, S.G., Mali, R.G. and Mehta, A.A. Effect of *Moringa oleifera* Lam. seed extract on Toluene Diisocyanate-induced immune-medicated inflammatory responses in rats. Journal of Immunotoxicology 2007b; 4 85–96.

[63] Hamza, A. A. Ameliorative effects of *Moringa oleifera* Lam seed extract on liver fibrosis in rats. Food and Chemical Toxicology 2010; 48 345-355.

[64] Siddiqui, S. and Khan, M.I. Pharmacological Study of *Moringa pterygosperma*. Central Laboratories. Pakistani Council of Science and Industrial Research 1968;p. 268–272.

[65] Kirtikar, K.R. and Basu, K. Indian Medicinal Plants. India: Lalit Mohan Basu MB, Allahabad; 1984. p.677–681.

[66] Nikkon, F., Saud, A., Rahman, M.H. and Haque, M.E. In vitro antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam. Pakistani Journal of Biological Sciences 2006; 6 1888–1890.

[67] Ghasi, S., Nwobodo, E. and Ofili, J.O. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed wistar rats. Journal of Ethnopharmacology 2000; 69 21–25.

[68] Dangi, S.Y., Jolly, C.I. and Narayanan, S. Antihypertensive activity of the total alkaloids from the leaves of *Moringa oleifera*. Pharmaceutical Biology 2002; 40, 144–148.

[69] Aruna, K., Sivaramakrishnan, V.M. Plant products as protective agents against cancer. Indian Journal of Experimental Biology 1990; 28 1008–1011.

[70] Diallo, D., Marston, A., Terreaux, C., Toure, Y., Paulsen, B.S. and Hostettmann, K. Screening of Malian medicinal plants for antifungal, larvicidal, molluscidal, antioxidant and radical scavenging activities. Phytotherapy Research 2001; 15 401–406.

[71] Rao, V.A., Devi, P.U. and Kamath, R. In vivo radioprotective effect of *Moringa oleifera* leaves. Indian Journal of Experimental Biology 2001; 39 858–863.

[72] Arora, R., Gupta, D., Chawla, R., Sagar, R., Sharma, A., Kumar, R., Prasad, J., Singh, S., Samanta, N. and Sharma, R.K. Radioprotection by plant products: present status and future prospects. Phytotherapy Research 2005; 19, 1–22.

[73] Cáceres, A., Cabrera, O., Morales, O., Mollinedo, P. and Mendia, P. Pharmacological properties of *Moringa oleifera*. 1. Preliminary screening for antimicrobial activity. Journal of Ethnopharmacology 1991; 33 213–216.

[74] Ruckmani, K., Kavimani, S., Anandan, R. and Jaykar, B. Effect of *Moringa oleifera* Lam. on paracetamol-induced hepatoxicity. Indian Journal of Pharmaceutical Sciences 1998; 60 33–35.

[75] Prakash, A.O. Ovarian response to aqueous extract of *Moringa oleifera* during early pregnancy in rats. Fitoterapia 1988; 59 89–96.

[76] Karadi, R.V., Gadge, N.B., Alagawadi, K.R. and Savadi, R.V. Effect of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. Journal of Ethnopharmacology 2006; 105 306–311.

[77] Rao, C.V., Ojha, S.K. and Mehrotra, S. Analgesic effect of *Moringa oleifera* leaf extract on rats. In: Proceedings of the Second World Congress on Biotechnological Developments of Herbal Medicine, Lucknow, India; 2003. p. 42.

[78] Choudhary, M.K.; Bodakhe, S.H. and Gupta, S.K. Assessment of the antiulcer potential of *Moringa oleifera* root-bark extract in rats. Journal of Acupuncture and Meridian Studies 2013; 6(4) 214-220.

[79] Asare, G.A.; Gyan, B.; Bugyei, K., Adjei, S.A., Mahama, R.; Addo, P., Otu-Nyarko, L., Wiredu, E.K. and Nyarko, A. Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation levels. Journal of Ethnopharmacology 2012; 139 265-272.

[80] Jideani, V. A. and Murevanhema, Y.Y. A Bambara groundnut milk beverage and probiotic yoghurt produced therefrom and a method for the production thereof. South Africa- Complete Patent 25 January 2013, 2013/00683.

[81] Jideani, V.A. & Diedericks, C.F. Dietary fibre from Bambara groundnuts (*Vigna subterranea*). South Africa Provisional Patent 14 June 2013, 2013/04395.

INTECH

Appendix C: Book of Abstracts – Research output presented at national and international conferences

1. **Diedericks, C.F.** & Jideani, V.A. (2014). Effect of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) soluble dietary fibre and orange oil in beverage emulsion stability. Paper presentation. In: Programme and Book of Abstracts – Research and Innovation for sustainable development. p. 24. U6 Consortium 2nd International Conference. Cape Town, South Africa.

2. **Diedericks, C.F.** & Jideani, V.A. (2014). Consumer Acceptance of White Bread Enriched with Bambara Groundnut (*Vigna subterranea* (L.) Verdc.) Insoluble Dietary Fibre. Moderated poster presentation. In: Book of Abstracts – Research That Resonates. IUFoST 17th World Congress of Food Science and Technology & Expo. MP7.3. Montreal, Canada.

3. **Diedericks, C.F.** & Jideani, V.A. (2013). Effect of Bambara groundnut (Vigna subterranea (L.) Verdc.) insoluble dietary fibre on physicochemical properties of white bread. Poster presentation. In: Celebration of Research Excellence Programme. p. 1. Cape Peninsula University of Technology Research Day. Cape Town, South Africa.

4. **Diedericks, C.F.** & Jideani, V.A. (2013). Some physical and hydration properties of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) insoluble dietary fibre. Paper presentation. In: Cape Peninsula University of Technology Postgraduate Conference. p. 13. Bellville, South Africa.

5. **Diedericks, C.F.** & Jideani, V.A. (2013). Physicochemical and functional properties of insoluble dietary fibre isolated from Bambara groundnut (*Vigna subterranea* (L.) Verdc.). Poster presentation. In: Book of Abstracts – Polysaccharides and polysaccharide-derived products, from basic science to applications. p. 274. EPNOE 2013 3rd International Polysaccharide Conference. Nice, France.

6. **Diedericks, C.F.** & Jideani, V.A. (2013). Potential of bambara groundnut (*Vigna subterranea* (L.) Verdc.) as dietary fibre food source. Poster presentation. In: Final Programme and Abstracts – Out of Africa: Global Food Science and Technology. p. 69. 20th SAAFoST Biennial International Congress and Exhibition. Pretoria, South Africa.

7. **Diedericks, C.F.** & Jideani, V.A. (2012). Underutilised legumes potential for Africa's food security. Poster presentation. In: Celebration of Research Excellence Programme. p. 2. Cape Peninsula University of Technology Research Day. Cape Town, South Africa.

8. **Diedericks, C.F**. & Jideani, V.A. (2012). Functionality of legume fibres in food and human physiology. Poster presentation. In: Cape Peninsula University of Technology Postgraduate Research Conference. p. 47. Bellville, South Africa.

9. **Diedericks, C.F.** & Felix-Minnaar, J. (2012). Effect of chicory (*Cichorium intybus* L.) root pulp addition on the functional properties of white bread. Poster presentation. In: Book of Abstracts. 16th IUFoST World Congress of Food Science and Technology. Brazil.

10. **Diedericks, C.F.** & Jideani, V.A. (2012). Potential of bambara groundnut (*Vigna subterranea* (L.) Verdc.) starch and non-starch polysaccharides as new food ingredients. Poster presentation. In: Book of Abstracts. 16th IUFoST World Congress of Food Science and Technology. Brazil.

11. **Diedericks, C.F.** & Jideani, V.A. (2011). Potential of bambara groundnut (*Vigna subterranea* (L.) Verdc.) starch and non-starch polysaccharides as new food ingredients. Poster presentation. In: Celebration of Research Excellence Programme. p. 1. Cape Town, South Africa.