CHARACTERISATION OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* (L.) VERDC.) NON-STARCH POLYSACCHARIDES FROM WET MILLING METHOD AS PREBIOTICS

YVONNE MAPHOSA

Thesis submitted in fulfilment of the requirements for the degree of

Master of Technology: Food Technology

in the Faculty of Applied Sciences

at the Cape Peninsula University of Technology

Supervisor: Professor Victoria A. Jideani

Bellville Campus

July 2015

DECLARATION

I, **Yvonne Maphosa**, 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.

Y. MAPHOSA **24/07/2015**

Signed **Date**

ABSTRACT

The aim of this study was to characterise the physicochemical, rheological, prebiotic and emulsion stabilising properties of four varieties (black-eye, brown-eye, brown and red) of Bambara groundnut (BGN) extracted using the modified wet milling method. A relatively high yield of BGN dietary fibres was obtained with soluble dietary fibres (SDFs) ranging from 15.4 to 17.1% and insoluble dietary fibres (IDFs) ranging from 12.0 to 15.6%. Black-eye and brown-eye dietary fibres showed superiority in terms of swelling capacities, water holding capacities, oil binding capacities, antioxidant properties as well as thermal stabilities than red and brown dietary fibres. In addition, black-eye and brown-eye dietary fibres were characterised by higher lightness (L*), redness (+a*), yellowness (+b*), chroma (C*) and hue. All four SDFs showed acceptable colour differences with $\Delta E < 8$ ranging from 0.81 to 3.08. The hydrolysable polyphenolic (HPP) content of SDFs ranged from 6.89 to 20.86 mg/g GAE and that of IDFs ranged from 10.96 to 14.43 mg/g GAE. All four SDFs differed significantly ($p < 0.05$) in their HPP content. BGN IDFs were very low in tannins (< 2.2 mg/g). Black-eye and brown-eye IDFs as well as brown and red IDFs did not differ significantly ($p > 0.05$) in their HPP content. Xylose, arabinose/galactose, mannose and rhamnose were the main sugars in IDFs, whilst mannose and xylose were the main sugars in SDFs. The sugar composition of BGN dietary fibres suggested the presence of galactomannans, arabinoxylan and arabinogalactans in SDFs and rhamnogalacturonans, cellulose, hemicellulose, arabinoxylans and low quantities of arabinogalactans in IDFs. The presence of these hydrocolloids in BGN dietary fibres could be an indication that BGN dietary fibres possess similar beneficial characteristics and thus can be classified in the same category as these hydrocolloids. The structural analysis of BGN dietary fibres showed SDFs having a Sauter mean diameter ($d_{3,2}$) ranging from 78.44 to 116.60 µm and a mean particle diameter ($d_{4,3}$) ranging from 74.89 to 124.29 µm. The Sauter mean diameter ($d_{3,2}$) of IDFs ranged from 78.44 to 120.87 µm and the mean particle diameter ($d_{4,3}$) ranged from 79.80 to 125.50 µm. All SDF solutions (4 - 14%) were pseudoplastic and thixotrophic with viscosity increasing with increasing concentration. All BGN dietary fibres showed high thermal stabilities and exhibited exothermic enthalpies. BGN SDFs were fermented by *B. breve, B. bifidum* and *B. animalis* subsp. *animalis* producing acetic and propionic acids as products of fermentation. The growth rates (μ_{max}) of *B. breve*, *B. bifidum* and *B. animalis* subsp. *animalis* were 0.33 to 0.46 Δ log cfu/mL/h, 0.46 to 1.75 Δ log cfu/mL/h and 0.54 to 1.75 Δ log cfu/mL/h, respectively. The growth rates of the three probiotics in BGN SDFs were significantly different ($p < 0.05$). Furthermore, BGN SDF stabilised orange oil beverage emulsion both in concentrated and diluted form. The emulsion stabilised with black-eye SDF had the highest droplet volume (86%) and lowest droplet size (≤3 µm). The volume-surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter ($d_{4,3}$) of the BGN SDF stabilised emulsions

ranged from 2.68-17.09 µm and 4.38-18.62 µm, respectively. Constrained non-linear regression protocol algorithms explained the turbidity loss rates of the emulsions and confirmed the stabilities of diluted BGN SDF stabilised emulsions. The outcome of this research revealed the potential of BGN dietary fibres as ingredients in various food systems.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to the following people and institutions that formed an integral part of the completion of this research study:

- Professor Victoria A. Jideani, my Supervisor and Senior Lecturer at the Cape Peninsula University of Technology, for being my source of inspiration and for her endless support and guidance throughout the duration of this study.
- Mr Fanie Rautenbach, Laboratory Manager in the Oxidative Stress Research Centre at the Cape Peninsula University of Technology, for his assistance with the polyphenolics and sugars analyses as well as freeze-drying.
- Mr Owen Wilson, Senior Technician in the Department of Food Technology at the Cape Peninsula University of Technology for assistance with procurement of chemicals and consumables.
- Mr. Ndumiso Mshicileli, Senior Technologist at the Agrifood Technology Station for assistance with Differential Scanning Calorimetry and Gas Chromatography analyses.
- Dr. Adeyi Oladayo, in the Department of Chemical Engineering at the Cape Peninsula University of Technology for assistance with rheology and emulsion analyses.
- Dimpho Ketso, Graduate Intern at the Agrifood Technology Station for assistance with some microbiological analyses.
- Stellenbosch University for assistance with equipment for Scanning Electron Microscopy.
- Cape Peninsula University of Technology University Research Funding (URF and National Research funding (NRF), for financial assistance towards the research running costs.

DEDICATION

Dedicated to my mother, Lesia Maphosa, for being such a phenomenal woman, for always picking me up when I'm down, for guiding me and always pushing me to be the best I can be, I love you mom. To my eldest brother Dr. Lancelot Maphosa, I look up to you. To my father Patrick David Maphosa, my siblings Mildred, Emmanuel, Lilian and Howard Ndabezinhle for being proud of me and for everything they have done for me. To Lovemore Nalube for always reminding me that I can do it. To Professor Victoria A. Jideani for being my source of inspiration and for being a wonderful supervisor. To all my friends, thank you for the endless support. And above all, to God almighty, for in Him I live, move and have my being, it is by His grace that I finished this work.

GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
AACC	American Association of Cereal Chemists
ADA	American Dietetic Association
ANOVA	Analysis of variance
BGN	Bambara groundnut
BS	Backscattering
$d_{3,2}$	Volume surface mean diameter
$d_{4,3}$	Equivalent volume-mean diameter
DF	Dietary fibre
DSC	Differential scanning calorimetry
FAO	Food and Agriculture Organisation
G’	Storage modulus
G’’	Loss modulus
GDP	Gross Domestic Product
HPP	Hydrolysable polyphenols
IDF	Insoluble dietary fibre
LSD	Least significant difference
MANOVA	Multivariate analysis of variance
NSP	Non-starch polysaccharides
OD	Optical density
O/W	Oil-in-water
PCA	Principal component analysis
RDI	Recommended daily intake
SWC	Swelling capacity
SCFA	Short chain fatty acids
SDF	Soluble dietary fibre
SEM	Scanning electron microscope
TDF	Total dietary fibre
USDA	United States Department of Agriculture
WHC	Water holding capacity
W/O	Water-in-oil

CHAPTER ONE
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Non-starch polysaccharides (NSPs) can be described as the indigestible portion of food derived mostly from plants (Khan *et al*., 2007). The terms, non-starch polysaccharides and dietary fibres, can therefore be used interchangeably. Definitions of fibre differ worldwide, with some based on a physiological basis and others on analytical methods (Slavin, 2013). Trumbo *et al*. (2003) stated that fibre can either be dietary or functional. Dietary fibre is made up of non-digestible carbohydrates that are inherent in plants (Elleuch *et al*., 2011) while functional fibre is made up of non-digestible carbohydrates that have a positive physiological effect to the human body (Trumbo *et al*., 2003).

Dietary fibre (DF) can be classified into two main components, namely soluble and insoluble fibres depending on the solubility in water. Insoluble dietary fibre (IDF) is the portion of DF that is not solvable in water and is metabolically inactive and soluble dietary fibre (SDF) on the other hand dissolves in water and is fermended in the human colon (Jenkins *et al*., 2002; Verma & Banerjee, 2010). Many fibre deficient food systems are fortified with DF from several sources to help elevate their nutritional value. The majority of foods consumed on a daily basis have low DF content, approximately 1 - 3% of DF per serving. A higher amount of DF is found in less popular foods such as whole grain cereals, dried fruits and legumes (Slavin, 2013).

A majority of fibres are prebiotic in nature and thus have received much attention from researchers (Slavin, 2013). A prebiotic is a food or food ingredient that has a positive impact to the host by supporting the growth of bacteria, such as *Bifidobacterium,* that is naturally resident in the colon (FAO, 2001; Fotiadis *et al*., 2008). The concept of prebiotics has received a lot of scientific and industrial interest since its introduction (Cummins *et al*., 2001; FAO, 2001; Gibson *et al*., 2004). Slavin (2013) reviewed the health outcomes of DF and prebiotics in humans and revealed the many beneficial effects these have on the human body. However, the researcher noted low intakes around the world which are below half of recommended daily intake (RDI).

The United States Department of Agriculture (USDA) and the United States Food and Drug Association recommend a daily intake of 25 g of DF for people of 14 years and older while the National Cancer Institute (America) and the American Dietetic Association (ADA) recommend 20 - 30 g/day and 20 - 35 g/day, respectively (Borderias *et al*., 2005; Verma & Banerjee, 2010; Biswas *et al*., 2011). The prebiotic nature of some DFs suggests that they can be used in the prevention and treatment of a broad spectrum of digestive complications (Baffoni *et al*., 2013). Dietary fibre from some legumes has been studied. However, the fibre

from Bambara groundnut (BGN) has not been widely studied, having only been reported by Diedericks (2014).

Bambara groundnut (BGN) (*Vigna subterranea* (L.) Verdc) is a legume belonging to the *Fabaceae* family grown in Africa, Asia, Australia, South and Central America (Jideani & Mpokotwane, 2009; Hillocks *et al.*, 2012; Jideani & Diedericks, 2014). In Africa, this legume is ranked as the third most significant legume following cowpeas and peanuts (Oyeleke *et al*., 2012). The production of BGN is very advantageous as it yields on adversely deficient soils, has nitrogen fixing ability and is drought, pest and disease resistant (Anon., 2011; Hillocks *et al.*, 2012). The pods of BGN ripen underground and when mature they can be consumed fresh or stored for consumption later on in the year (Olaleye *et al*., 2013). The dried seeds are difficult to cook requiring large amounts of time; this is believed to be one of the limitations to its increased utilisation (Olaleye *et al*., 2013). Nutritionally, BGN is qualified as a complete food product by several researchers (Ajayi & Lale, 2001; Murevanhema & Jideani, 2013). These researchers reported the composition of BGN as 55 - 70% carbohydrates, 6 - 9% fat, 17 - 25% protein, 5.2 - 6.4% dietary fibre (DF) and an appreciable amount of micronutrients. Considering the RDIs of DF reported by Slavin (2013), the DF content in BGN is sufficient to significantly improve the fibre content in fibre deficient foods. The nutritional value of BGN suggests that this legume can play a major role in reducing malnutrition and providing an affordable source of nutrients (Turner & Lupton, 2011). The increased exploitation of BGN has the potential to lessen the over-utilisation of other sources of nutrients such as peas, lentils and soybeans.

Dietary fibre can be extracted from leguminous crops using either wet or dry methods. Most of the methods make use of the enzymatic-chemical combination or the enzymatic-gravimetric combination (McCleary *et al*., 2011). The basis of most of the methods commonly used is hydrolysis and elimination of other proximate components leaving behind DF (Anon., 2010). Most of the methods used lack robustness, are relatively expensive and require many chemicals (McCleary *et al*., 2011). Wet milling utilises less water than other wet methods and requires minimal chemicals (Dalgetty & Baik, 2003). A cost effective method of DF extraction from BGN seeds is essential.

1.2 Statement of the Research Problem

The therapeutic, prebiotic, functional, physiological and nutritional properties of dietary fibres have encouraged the growth of a market of fibre rich products, consequently leading to a demand for new DF sources. Legumes are generally high in DF. Research has been carried out on the extraction and utilisation of dietary fibres from various legumes such as peas and lentils. Dietary fibre from BGN was investigated by Diedericks (2014); however a relatively costly enzymatic-gravimetric method was employed in the study. As such, a cost effective, reproducible and easy-to-handle method of extracting DFs from BGN remains

unreported. The extraction and characterisation of BGN DFs could play a role in increasing the value of this legume as well as bridge the gap in the market by increasing its utilisation. The prebiotic effects of BGN DFs have also not been reported. An understanding of the use and fermentation of BGN DFs by *Bifidobacterium* is needed to scientifically validate any prebiotic properties and the subsequent health benefits the DFs may afford.

1.3 Objectives of the Study

1.3.1 Broad objective

The broad objective of this research was to evaluate Bambara groundnut non-starch polysaccharides from wet milling method with the view to establish their physicochemical, rheological, prebiotic and emulsion stabilising properties.

1.3.2 Specific objectives

The specific objectives of this research included the following:

1. Extract and quantify soluble and insoluble dietary fibres from four BGN varieties (black-eye, brown-eye, brown, red) using the wet milling method.
2. Compare the cost of the wet milling and the enzymatic-gravimetric methods as reported by Diedericks (2014).
3. Determine the physicochemical, thermal, rheological and structural properties of the BGN DFs.
4. Investigate the ability of BGN soluble dietary fibre to support the growth of *Bifidobacterium breve, Bifidobacterium bifidum* and *Bifidobacterium animalis* subspp*. animalis in vitro*.
5. Determine whether BGN soluble dietary fibres stabilise emulsions under storage conditions at room temperature (23 - 25°C).

1.4 Hypotheses

The hypotheses that were tested in this research were:

1. The extraction of soluble and insoluble fibres from BGN will be possible using the wet milling method.
2. BGN SDFs will sufficiently stabilise beverage emulsions and significantly affect their rheological properties.
3. The wet milling method will be relatively cheaper and simpler than the enzymatic-gravimetric method while producing fibres of similar quality.
4. The soluble dietary fibres will support the growth of *Bifidobacterium breve, Bifidobacterium bifidum* and *Bifidobacterium animalis* subspp*. animalis*.

1.5 Delimitations

The delimitations of this research were that:

1. Only the wet milling method was used in the extraction of BGN dietary fibre.
2. Only four varieties of BGN namely the black-eye, brown-eye, brown and red were used.
3. *Bifidobacterium breve, Bifidobacterium bifidum and Bifidobacterium animalis subspp. animalis* were the only probiotics studied.

1.6 Significance of the Research

This research will generate knowledge of the chemistry of BGN non-starch polysaccharides. In addition, it will reveal the potential of BGN as a source of dietary fibre and a potential prebiotic. The study will reveal the potential of fibre extracted from BGN for commercialisation. Successful commercialisation would, in the long run, lead to the requirement of more BGN fibre. This would encourage farmers to increase the production of BGN and thus empowering local farmers including rural farmers as they too would be able to commercialise the BGN they cultivate. An increase in the production of BGN would increase the demand for manpower thus creating employment resulting in improved living conditions of previously unemployed people. An increase in production and the availability of knowledge of the benefits of BGN will also result in the economic exportation of the legume to generate wealth and livelihoods.

Bambara groundnut is commonly cultivated by women in arid and semi-arid regions, especially in sub-Saharan Africa where this legume is popular. Hence an increase in its production would also empower women and improve their socio-economic status. Furthermore, successful commercialisation and the resultant increase in the cultivation of BGN would help to alleviate malnutrition and increase food security.

1.7 Outcomes, Results and Contributions of the Research

Insoluble and soluble dietary fibres from BGN were produced from this study. The investigation of the physicochemical properties of BGN fibres generated information on the nature, use and functions that these fibres could be subjected to. The *in vitro* prebiotic studies provided a better understanding of the behaviour of the BGN fibres in the human colon. Results of the stabilising and rheological effects of BGN fibres on beverage emulsions gave insight into the instability and shelf-life of the systems both in concentrated and diluted forms. Rheological assessments offered a better understanding of the nature of the fibres, their viscous and viscoelastic properties as well as their behaviour under varying stresses. The results of this study enabled an accurate prediction of applications to which BGN fibres could be put to. Furthermore, a review article from this work was published in Food Reviews International and a research article was published in the South African

Journal of Science. Two other research articles are under consideration for publication in the South African Journal of Science and the African Journal of Science, Technology, Innovation and Development. Findings from this study were presented at U6 Consortium 2nd International Conference, Cape Town, South Africa (2014), at the Cape Peninsula University of Technology Post Graduate Conference (2014), at the Institute of Food Technologists, IFT15 International Conference, Chicago, Illinois, USA (11 – 14 July 2015) and at the 21st South African Association of Food Science and Technology (SAAFoST) Biennial International Congress and Exhibition, Durban, South Africa (6 – 9 September 2015). The attainment of a Master of Technology degree was also expected from this study.

1.8 Thesis Overview

This thesis consists of six chapters and was structured in article format where each chapter is an individual entity. Figure 1.1 shows the structure of the thesis. Chapter one gives the motivation and design of the study, stating the research problem, objectives, hypotheses, delineation of the study, significance of the research as well as expected outcomes, results and contributions of the research. Chapter two is literature review. A background on Bambara groundnut (BGN) was given, including its nutritional composition, various uses, market potential and its future prospects. The definitions, components, health benefits, relevance for the food industry, extraction and fractionation of dietary fibre from various sources as well as its physicochemical characteristics and recommended intakes were outlined. Furthermore, prebiotics and probiotics, with emphasis on *Bifidobacterium*, were reviewed. Finally, the stability and application of emulsions in food systems as well as rheology were briefly reviewed.

Chapter three is the first research chapter detailing the isolation of soluble and insoluble dietary fibres from the black-eye, brown-eye, brown and red varieties of BGN and their physicochemical properties. The rheological properties of 4 - 14% solutions of BGN soluble dietary fibres were also evaluated in Chapter three. The time dependent, time independent and oscillatory behaviours of the soluble dietary fibre (SDF) solutions were assessed and the data modelled using Power law. Chapter four is the second research chapter focusing on the prebiotic characteristics of the four BGN soluble dietary fibre varieties using *B. breve, B. bifidum* and *B. animalis* subspp*. animalis* as probiotics. Batch culture fermentation systems were used in the research. Optical densities, direct microscopical counts and short chain fatty acid production were determined in the assessment of the prebiotic properties of BGN fibres. Gompertz kinetic parameters were

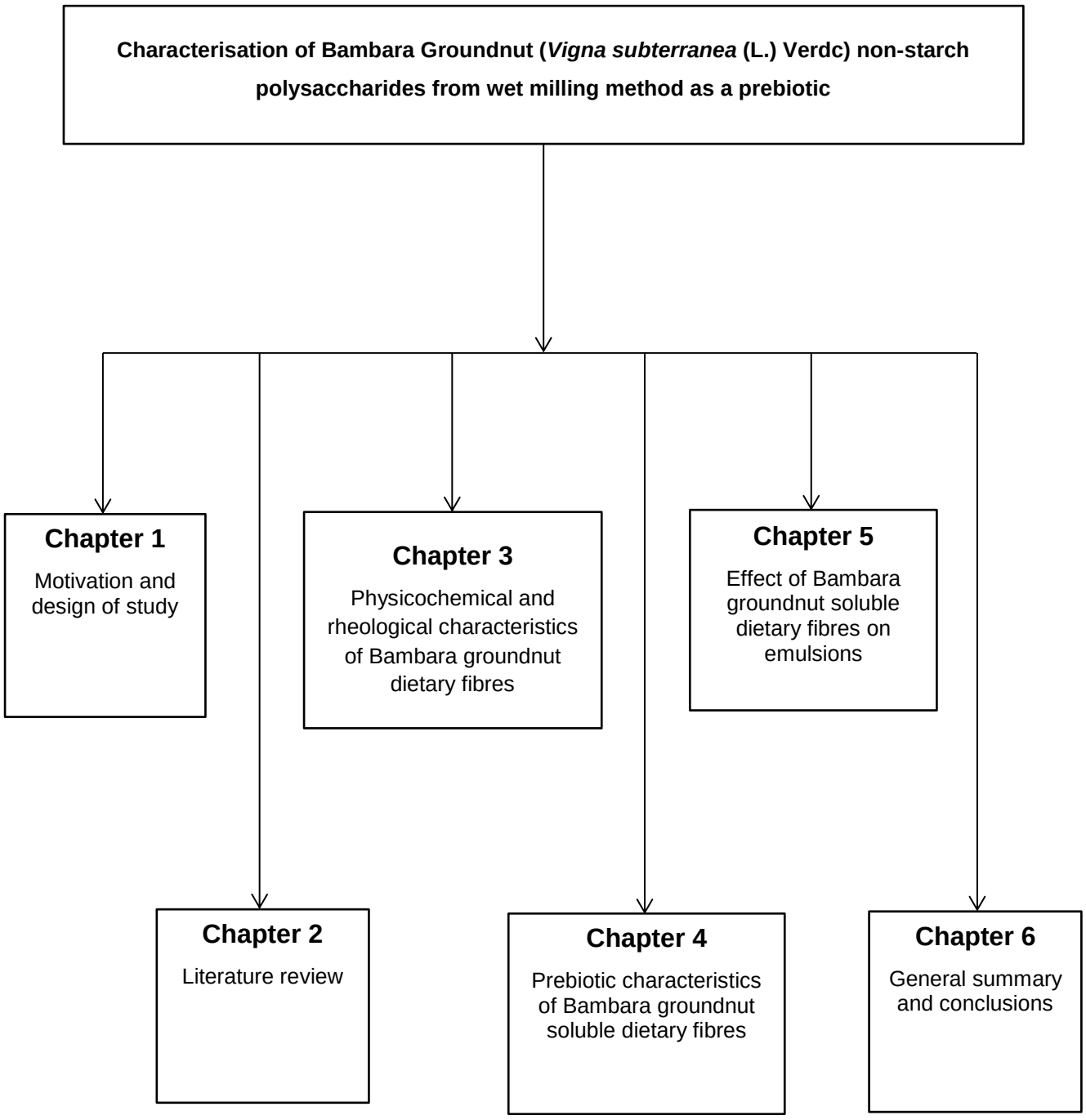

Figure 1.1 Thesis overview.

used to explain the growth characteristics of *Bifidobacterium* spp. in the presence of BGN SDFs.

Chapter five is the third research chapter focusing on the stability, structure and rheological properties of emulsions formulated with 30% soluble dietary fibre from the four BGN varieties and 6% orange oil. Stability of concentrated emulsions was evaluated using Turbiscan and that of diluted emulsions was assessed by determining the turbidity loss rates of the emulsions stored at room temperature (23 - 25°C) by spectrophotometry. Constrained non-linear regression was applied in modelling turbidity loss rates of emulsions. Droplet size and droplet size distribution as well as microstructural images gave information on the structure of the emulsions. The time dependent, time independent and oscillatory behaviours of the emulsions were also assessed and modelled using Power law. Chapter six is a summary of the research, giving general conclusions of the study.

References

Ajayi, F. A. & Lale, N.E.S. (2001). Susceptibility of unprotected seed and seeds of local Bambara groundnut cultivars protected with insecticidal essential oils to infestation by *Callosobruchusmaculates. Journal of Stored Products Research*, **37**, 47 - 62.

Anonymous. (2010). Processing methods for dry peas, lentils & chickpeas. In: *Processing Information & Technical Manual*. Moscow: USA Dry Pea & Lentil Council.

Anonymous. (2011). Department of Agriculture, Forestry and Fisheries. Directorate Production/ Plant Production. [Internet document] URL: http://www.nda.agric.za/docs/Brochures/ProdguideBambara.pdf. Accessed (25/01/2014).

Baffoni, L., Stenico, V., Strahsburger, E., Gaggìa, F., Gioia, D.D., Modesto, M., Mattarelli, P. & Biavati, B. (2013). Identification of species belonging to the *Bifidobacterium* genus by PCR-RFLP analysis of a *hsp60* gene fragment. *BMC Microbiology*, **13**, 149.

Biswas, A.K., Kumar, V., Bhosle, S., Sahoo, J. & Chatli, M.K. (2011). Dietary fibers as functional ingredients in meat products and their role in human health. *International Journal of Livestock Production*, **2**(4), 45 - 54.

Borderias, A.J., Sanchez-Alonso, I. & Perez-Mateos, M. (2005). New applications of fibres in foods: addition to fishery products. *Trends in Food Science and Technology*, **16**, 458 - 465.

Cummings, J.H., Macfarlane, G.T. & Englyst, H.N. (2001). Prebiotic digestion and fermentation. *American Journal of Clinical Nutrition*, **73**, 415 - 420.

Dalgetty, D.D. & Baik, B. (2003). Isolation and characterisation of Cotyledon Fibers from Peas, Lentils, and Chickpeas. *American Association of Cereal Chemists*, **80**(3), 310 - 315.

Diedericks, C.F. (2014). Functional properties of Bambara groundnut (*Vigna Subterranea* (L) Verdc.) non-starch polysaccharides in model and food systems. Master of Technology: Food Technology Thesis, Cape Peninsula University of Technology.

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

FAO. (2001). Food and Agriculture Organization of the United Nations. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Pp 20.

Fotiadis, C.I., Stoidis, C.N., Spyropoulos, B.G. & Zografos, E.D. (2008). Role of probiotics, prebiotics and synbiotics in chemoprevention for colorectal cancer. *World Journal of Gastroenterology,* **14**(42), 6453 - 6457.

Gibson, G.R., Probert, H.M., Loo, J.V., Rastall, R.A. & Roberfroid, M.B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews*, **17**, 259 - 275.

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

Jenkins, D.J.A., Kendall, C.W.C., Vuksan, V., Vidgen, E., Parler, T., Faulkner, D., Mehling, C.C., Garsetti, M., Testolin, G., Cunnane, S.C., Ryan, M.A. & Corey P.N. (2002). Soluble fiber intake at a dose approved by the US Food and Drug Administration for a health claim of health benefits: Serum lipid risk factors for cardiovascular disease assessed in a randomized controlled crossover trial. *American Journal of Clinical Nutrition,* **75**, 834 - 839.

Jideani, V.A. & Mpotokwane, S.M. (2009). Modelling of water absorption of Botswana Bambara varieties using Peleg's equation. *Journal of Food Engineering*, **92**(2), 182 - 188.

Jideani, V.A. & Diedericks, C.F. (2014). Dietary Fibre Supplement. South Africa complete patent (2014/04371).

Khan, A.R., Alam, S., Ali, S., Bibi, S. & Khalil, I.A. (2007). Dietary fiber profile of food legumes. *Sarhad Journal of Agriculture*, **23**(3), 763 - 766.

McCleary, B.V., DeVries, J.W., Rader, J.I., Cohen, G., Prosky, L., Mugford, D.C., Champ, M. & Okuma, K. (2011). Collaborative study report: Determination of Insoluble, Soluble and Total Dietary Fiber (Codex definition) by an enzymatic-gravimetricmethod and liquid chromatography. *AACC International Report*, **56**(6), 238 - 247.

Murevanhema, Y.Y. & Jideani, V.A. (2013). Potential of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk as a probiotic beverage - A review. *Critical Reviews in Food Science and Nutrition*, **53**(9), 954 - 967.

Olaleye, A.A., Adeyeye, E.I. & Adesina, A.J. (2013). Chemical composition of bambara groundnut (*V. subterranea* L. Verdc) seed parts. *Bangladesh Journal of Scientific and Industrial Research*, **48**(3), 167 - 178.

Oyeleke, G.O., Afolabi, O. & Isola, A.D. (2012). Some Quality Characteristics and Carbohydrate Fractions of Bambara Groundnut (*Vigna subterranea* L.). Seed Flour. *Journal of Applied Chemistry*, **2**, 16 - 19.

Slavin, J. (2013). Fiber and Prebiotics: Mechanisms and Health Benefits. *Nutrients*, **5**(4), 1417 - 1435.

Trumbo, P., Schlicker, S., Yates, A.A. & Poos, M. (2003). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *Journal of American Dietetic Association*, **103**(5), 563.

Turner, N.D. & Lupton, J.R. (2011). Dietary fiber. *Advances in Nutrition: An International Review Journal,* **2**, 151 - 152.

Verma, A.K. & Banerjee, R. (2010). Dietary fibre as functional ingredient in meat products: a novel approach for healthy living – A review. *Journal of Food Science and Technology*, **47**(3), 247 - 257.

CHAPTER TWO
LITERATURE REVIEW

2.1 Bambara Groundnut

Bambara groundnut (BGN) scientifically known as *Vigna subterranea* (L.) Verdc is a legume predominantly grown in sub-Saharan Africa (Madukwe *et al.*, 2011; Jideani & Diedericks, 2014a). It is cultivated mainly by females on a small scale for domestic purposes (Mpotokwane *et al.*, 2008). It is a neglected legume that is not frequently consumed due to the long time it takes to cook the dried seeds as well as the insufficient knowledge on ways of utilizing the seeds (Fasoyiro *et al.*, 2012). Bambara groundnut is easy and cheap to cultivate, yields well in mineral deficient soils and is drought, pest and disease resistant (Mwale *et al*., 2007; Diedericks, 2014). These advantages of BGN as well as its many beneficial attributes are known, however its agro-ecological and commercial potential have not been fully investigated (Azam-Ali *et al*., 2001a). The underutilisation of BGN may be attributed to the perception that it is a crop of low economic value (Azam-Ali *et al*., 2001b). Other probable reasons for its underutilisation could be due to farmers giving priority to other food crops perceived to have a larger market value such as maize (Shanmugasundaram, 2003).

The Department of Agriculture, Forestry and Fisheries reported seven varieties of BGN namely the red, black, brown, cream/black-eye, cream/brown-eye, cream/no-eye, and the speckled/flecked/spotted variety (Anon., 2011a) as shown in Table 2.1. The red variety is late maturing with large kernels, however, it is prone to rotting onsite. The black variety is early maturing, mainly one seeded with small to medium-sized kernels. The kernels of the brown variety are medium to large sized and their colour varies between light and dark brown. The cream/black-eye variety has large kernels and the cream/brown-eye has moderately sized kernels. The cream/black-eye and cream/brown-eye varieties are all good yielders. The cream/no-eye variety has very small pods and kernels and mainly produces one seed and its yields are relatively lower. The purple colour predominates in the speckled/flecked/spotted variety; the variety also has one seeded pods and small kernels (Anon., 2011a). Figure 2.1 shows different varieties of Bambara groundnut.

2.1.1 Nutritional composition of Bambara groundnut

Bambara groundnut is qualified as a complete nutritious food product (Ajayi & Lale, 2001; Shanmugasundaram, 2003; Afoakwe *et al.*, 2007; Qayyum *et al*., 2012). Table 2.2 gives the nutritional composition of BGN. BGN has a high nutritional value with carbohydrates, proteins and dietary fibre in the range 55 – 70%, 17.0 – 25.0% and 5.2 – 6.4%, respectively.

Table 2.1 Classification of Bambara groundnut

Family	***Fabaceae***
Genus	*Vigna*
Species	*Vigna subterranean*
Scientific name	*Vigna subterranea* (L.) Verdc
Varieties	*V. subterranea var*. *Spontanea* (wild variety)
	V. subterranea var. Subterranea (cultivated variety)
Common name	Bambara groundnut
Varieties	Red
	Black
	Brown
	Cream/black-eye
	Cream/brown-eye
	Cream/no-eye
	Speckled/flecked/spotted

(Adapted from: Anon., 2011a)

a

b

c

d

e

Figure 2.1 Different varieties of Bambara groundnut seeds: (a) black-eye (b) brown-eye, (c) mixture of varieties (d) red (e) brown (Diedericks, 2014).

Table 2.2 Nutritional composition of Bambara groundnut

Nutrient	Amount (%)
Fat	6.0 – 9.0
Carbohydrates	55.0 – 70.0
Protein	17.0 – 25.0
Dietary fibre	5.2 - 6.4
Potassium	1.6
Magnesium	0.2
Calcium	0.9
Nitrogen	3.9
Phosphorus	0.6

(Adapted from: Baryeh 2001; Mkandawire, 2007; Murevanhema & Jideani, 2013)

In addition, BGN also contains an appreciable amount of minerals such as potassium, magnesium, calcium, nitrogen and phosphorus as shown in Table 2.2. The red variety specifically, has almost twice the amount of iron as the cream varieties and hence could be useful in curbing iron deficiency (Hillocks *et al.*, 2012). According to Kone *et al.* (2011) BGN seeds contain enough quantities of proteins, carbohydrates and lipids to sustain human life. The protein of BGN is richer in the essential amino acids, methionine and lysine, compared with most grain legumes (Murevanhema & Jideani, 2013). Thus, nutritionally, BGN is unique in providing the essential amino acids that are deficient in most leguminous crops. The high protein content of BGN complements cereals and is an excellent source of proteins for vegetarians and lower class individuals who cannot afford meat derived protein (Baryeh, 2001; Guillon & Champ, 2002; Massawe *et al.*, 2005; Bamshaiye *et al.*, 2011; Mabhaudhi *et al*., 2013). BGN is also naturally cholesterol-free and low in saturated fatty acids (Shanmugasundaram, 2003). The increased utilisation of BGN holds great promise to increase food security, alleviate malnutrition and offer potential of cheaper nutritious products (Shanmugasundaram, 2003; Mabhaudhi *et al*., 2013).

2.1.2 Various uses and yield of Bambara groundnut

Bambara groundnut is mainly cultivated for human consumption (Jideani & Diedericks, 2014a). Apart from food applications, some cultures believe that BGN has medicinal properties and can be used in traditional ceremonies such as funerals and gift exchanges (Shegro *et al.*, 2013). The various food uses of BGN are given in Table 2.3. BGN seeds are utilised mostly at household level. The fresh or dry seeds are boiled or roasted and consumed on their own or are incorporated in other dishes.

The total area that most peasant farmers designate for the cultivation of BGN is very low, compared to other crops such as maize, peanuts and sorghum (Shanmugasundaram, 2003). The yield of BGN under traditional farming conditions was estimated to be in the range 300 - 850 kg/ha with expected yields of less than 100 kg/ha in some fields (Baudoin & Mergaeai, 2001; Baryeh, 2001). Brink *et al*. (2006) reported the highest seed yield under field conditions as 4 t/ha. In 1972, the world production of BGN was said to be at 29800 tonnes (FAO, 2011), in 1980 it increased to an estimated 33000 tonnes (Ajayi & Lale, 2001) and was at 79155 tonnes in 2005 (FAO, 2011). Since 2005 to the current date the yield of BGN has not increased significantly. This serves as an indication that BGN has not received sustained attention as evidenced by no yield increases.

This research aims to provide knowledge of BGN and the beneficial effects of its constituents, particularly its DF and hence increase commercialisation which if successful will in turn increase production.

Table 2.3 Various food uses of Bambara groundnut

Form	Uses	Source
Mature, fresh or dry seeds	Eaten as pulse	Brink *et al.* (2006)
Dried seeds (whole or split)	Boiled with maize or plantains	Lim (2012)
Dried seeds (ground)	Added to maize flour for porridge	Brink *et al.* (2006)
Soaked and ground seeds	Paste for fried or steamed dishes	Brink *et al.* (2006)
Boiled salted seeds	Appetisers	Lim (2012)
Dried seeds	Commercially canned in gravy	Lim (2012)
Dried and boiled	Used in soups	Murevanhema & Jideani (2013)
Crushed, roasted seeds	Relish	Lim (2012)
Flour and milk derived from BGN	Yoghurt beverages, bread	Murevanhema & Jideani (2013)
Testa-free fresh seeds	Consumed as a complete meal by cooking with seasoning	Jideani & Diedericks (2014a)
Dry BGN seeds	Boiled and crushed seeds used to form cakes/ balls followed by frying and adding to stews	Jideani & Diedericks (2014a)

2.1.3 Market potential and future prospects of Bambara groundnut

Despite having nutritional advantages over other commercial legumes, BGN has not developed as a traded commodity. Hillocks *et al*. (2012) suggested that this could be due to insufficient demand in the formal market resulting from lack of marketing and promotion. Empirical evidence and the results of various researches have revealed the potential of BGN both as a crop and as food (Lawal *et al*., 2004; Jideani & Murevanhema, 2012; Gabriel *et al.*, 2013; Adeyi, 2014; Jideani & Diedericks, 2014b). This crop deserves more publicity and increased utilisation than it is currently receiving. Bambara groundnut is a low budget, low cost, reliable crop that thrives in adverse conditions too harsh for other crops (Hillocks *et al.*, 2012). The public, especially the lower income groups, need to be educated on the nutritional value of BGN.

Murevanhema (2012) successfully studied the production of a probiotic beverage and yoghurt from BGN. This beverage was found to have potential as an alternative for lactose free milk in the market and the yoghurt had probiotic effects which would have positive health effects on consumers. The production of a probiotic beverage from BGN flour proved to be energy saving and thus would result in an affordable product in the market place. In addition, the beverage was characterised by a high phenolic and flavonoid content which would contribute to nutritional well-being (Murevanhema, 2012). Sensory evaluation indicated that the probiotic beverage and yoghurt would be widely accepted in the market by all age groups. Notable is the work of Jideani & Murevanhema (2012) who patented a process for the production of a prebiotic beverage from BGN flour.

Hillocks *et al.* (2012) observed that the addition of boiled BGN to fermented maize dough increased its protein content from 10.0 - 16.4%. BGN flour can be utilised by the cereal and bakery industry as a wheat replacement in biscuit and flat cakes as suggested by Hillocks *et al.* (2012). Diedericks (2014) studied the effect of BGN insoluble fibres from black-eye, brown-eye, red and brown varieties on white bread. BGN insoluble fibres significantly increased the dietary fibre content of the bread, lowered the specific loaf volume and had positive textural effects especially on crumb softness. As such, BGN insoluble fibres would make good ingredients and fortifying agents in the bakery industry. Jideani & Diedericks (2014b) patented the extraction of dietary fibre from BGN seeds using the enzymatic-gravimetric method.

Adeyi (2014) studied the effect of BGN flour on the stability and rheology of oil-in-water emulsions and reported a high stability associated with emulsions stabilised with BGN flour. BGN flour was shown to positively influence the time dependent, time-independent and oscillatory characteristics of the studied oil-in-water emulsions. The emulsifying properties of BGN flour and starch on oil-in-water emulsions were reported by Gabriel *et al.* (2013). The researchers reported BGN flour as a better emulsifier than BGN starch. BGN

flour was reported to significantly reduce the migration rate of oil droplets as well as reduce the droplet size of the emulsion hence resulting in higher stability.

The mentioned studies indicated the potential of BGN for use in different branches of the food industry. Efforts should be intensified to improve the utilisation of BGN in Africa and the rest of the world. There is a considerable need for new and innovative uses of BGN. The challenge is to narrow the gap and improve food security at household level. To attract the attention of consumers, innovative ways of presenting BGN as a food or food ingredient are required (Diedericks, 2014). Innovation may include the production of snacks from BGN, marketing BGN as a prebiotic and extracting the constituents of BGN such as dietary fibre for inclusion in other food applications.

2.2 Dietary Fibre

2.2.1 Definitions of dietary fibre

Dietary fibre is difficult to define because it includes a wide range of complex compounds (Verma & Banerjee, 2010). Traditionally DF has been defined as a mixture of polymeric non-starch polysaccharides such as cellulose, hemi-cellulose and pectin, that are not digestible by enzymes in the gastro-intestinal canal (Guillon & Champ, 2000; Borderias *et al*., 2005; Khan *et al*., 2007). This definition is analogous to that given by Garcia *et al*. (2007) and Elleuch *et al*. (2011) who defined DF as non-digestible carbohydrate and lignin that are components of plants including the non-digestible carbohydrates that have beneficial functional effect in the human body. The definition has been broadened to include fibres derived from animals such as chitosans and modified or synthetic non-digestible polysaccharides as part of the definition (Borderias *et al*., 2005; Elleuch *et al*., 2011). The Codex Alimentarius Commision (2009) defined dietary fibre as carbohydrate polymers consisting of more than nine monomers, which are not digestible by the intestinal enzymes and could be classified under the following categories:

1. Fit for consumption carbohydrate polymers that are inherent in the food as consumed
2. Synthetic carbohydrate polymers which have been scientifically proven to have a functional effect to the human body
3. Carbohydrate polymers attained from foodstuff by physical, enzymatic or chemical means and which have been scientifically proven to have a functional effect to the human body

The Europeon Commision (2006) shared the same definition as Codex (2009) except that their definition included carbohydrate polymers with three or more monomers (Westenbrink *et al*., 2012). Definitions of fibre differ worldwide, some are based on a physiological basis and some on analytical methods applied for analysis (Westenbrink *et al*., 2012; Slavin, 2013). The American Association of Cereal Chemists (AACC, 2001) defined dietary fibre as the fragments of plants or the saccharides that are not digested in the

intestines and are partially or completely fermented in the colon (Dhingra *et al*., 2012). The AACC further stated that these saccharides promote overall health by playing a role in the reduction of blood sugar and cholesterol (AACC, 2001).

Considering the different definitions discussed above, dietary fibre can be defined as any polymeric non-starch polysaccharide of plant or animal origin, whether natural, synthetic or modified, that resists digestion in the small intestines and is fermentable by resident microbiota in the colon and whose fermentation has a beneficial effect on the human body.

2.2.2 Components of dietary fibre

Dietary fibre is composed mainly of parts of the cell wall (Figuerola *et al*., 2005; Huang *et al*., 2011; Westenbrink *et al*., 2012). Plant cell walls are made predominantly of polysaccharides which include cellulose, hemicelluloses, pectin, gums, inulin and resistant starches (Figuerola *et al*., 2005; Biswas *et al*., 2011; Elleuch *et al*., 2011). Cellulose is a major component of plant cells, composed of linear glucose chains (Figure 2.2a) joined together by β 1-4 glycosidic bonds (Mwaikambo, 2006; Khan *et al*., 2007). Due to the extensive hydrogen bonding within cellulose microfibrils, cellulose is rendered insoluble and has high mechanical strength (Dhingra *et al*., 2012). Pectic substances (Figure 2.2b) are characterised by α 1-4 linked polymers of D-galacturonic acid units esterified with methanol (Biswas *et al*., 2011). Lignins are complex molecules of polyphenylpropane units (Figure 2.2c). Lignins and pectin constitute only a small portion of DF (Khan *et al*., 2007). Hemicelluloses (Figure 2.2d) are commonly found in the seed endosperm particularly in guar. Some hemicelluloses such as galactomanans are water soluble, consisting of about 63% mannose and 35% galactose. However, most of them are linear xylose polymers with glucoce, arabinose and glucuronic acid side chains (Khan *et al*., 2007).

Fibre can be classified as dietary or functional and can further be classified according to its molecular weight as high molecular weight DF or low molecular weight DF (Trumbo *et al*., 2003; Westenbrink *et al*., 2012). Dietary fibre can also be classified according to its water solubility as soluble dietary fibre (SDF) or insoluble dietary fibre (IDF) (Prakongpan *et al*., 2002). Lignin, cellulose and some hemicellulose typically make up the bulk of IDF while pectin, some hemicellulose and other non-starch DF polysaccharides make up SDF (Khan *et al.*, 2007).

Insoluble dietary fibre is the portion of DF that does not dissolve in water and is commonly derived from cereals (Verma & Banerjee, 2010). Soluble dietary fibre on the other hand dissolves in water and is fermented in the large intestines. Fruits and vegetables are the common sources of SDF (Jenkins *et al*., 2002). The ratio of SDF to IDF, particle size and source of the fibre are some of the attributes of importance for both functional and dietary properties (Figuerola *et al*., 2005).

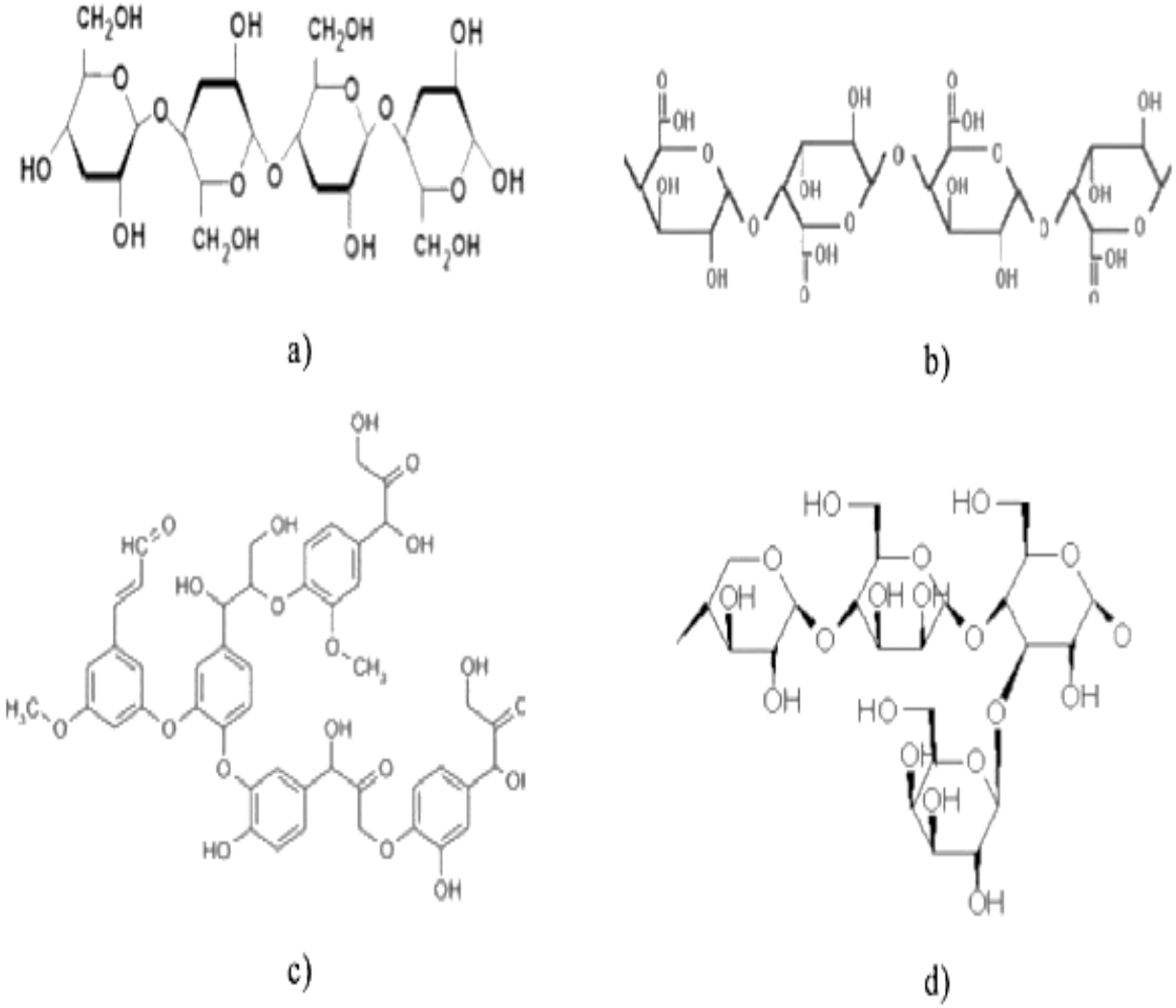

Figure 2.2 Structure of (a) cellulose (b) pectin (c) lignin (d) hemicelluloses (Source: Ophardt, 2003; Coultate, 2009; Vanholme *et al.*, 2010, Dhepe & Sahu, 2010).

A ratio of approximately 1:2 of SDF: IDF is considered acceptable for dietary fibre destined for use as a food ingredient (Figuerola *et al*., 2005). Characteristics of commercial fibre according to Figuerola *et al*. (2005) are a total dietary fibre content of above 50%, a moisture content of less than 9%, a very low lipid and calorie content and a neutral flavour and taste.

2.2.3 Health benefits of dietary fibre

Adequate daily intake of DF is largely recommended (Guillon & Champ, 2002; Wood & Grusak, 2007). Published reports indicate the many beneficial effect of DF in the human body (Figure 2.3). These include the prevention and possible treatment of diseases and disordconditions like constipation, obesity, diabetes, heart complications, piles and some cancers (Chau & Huang, 2004; Khan *et al*., 2007; Huang *et al*., 2011). In addition, DF, particularly SDF has the ability to lower blood cholesterol, improve glucose tolerance and reduce glycaemic response by forming a protective gel lining along the intestinal walls thus reducing glucose and cholesterol assimilation into the bloodstream (Hawkes, 2006; Khan *et al*., 2007; Garcia *et al*., 2007). Insoluble dietary fibres are porous, have low densities, increase faecal bulk and promote normal laxation (Fernandez-Lopez *et al*., 2004; Rosell *et al*., 2009; Elleuch *et al*., 2011).

Over the years, consumers have become more concerned about the effect of diet on health since nutrition is associated with many diseases of lifestyle such as diabetes, obesity and some cancers (Cava *et al*., 2012). This includes the increased awareness of the beneficial health attributes and nutritional significance of DF which has resulted in a higher demand of high fibre foods (Choi *et al*., 2014). In an effort to meet this demand, the investigation of substitute sources of DF by some researchers has been carried out (Dalgetty & Baik, 2003; Chau & Huang, 2004; Khan *et al*., 2007; Ojimelukwe, 2009).

The extraction of DF, particularly from legumes, has received attention from various researchers (Khan *et al*., 2007; Diedericks, 2014). The DF content of most edible legumes ranges from 8.0 - 27.5%, with SDFs in the range 3.3 - 13.8%. Guillon & Champ (2002) reported DFs ranging from 6.9 - 9.3% for pea, broad pea and soybean cotyledons. Literature reveals that legume seeds such as BGN have a higher amount of DF, up to 24.3%, than cereals and are sources of metabolically active SDF (Fasoyiro *et al*., 2006; Khan *et al.*, 2007; Diedericks, 2014).

The DF content of BGN has been documented, however variations in the reported results exist. These variations could be due to differences in BGN varieties, species, climatic conditions, type of soil grown on, processing and determination methods used (Guillon & Champ, 2002; Khan *et al*., 2007; Murevanhema, 2012). Murevhanema & Jideani (2013) reported the BGN DF content as ranging from 5.2 - 6.4% while Diedericks (2014) reported BGN DF in the range 17.7 – 24.3% dry matter.

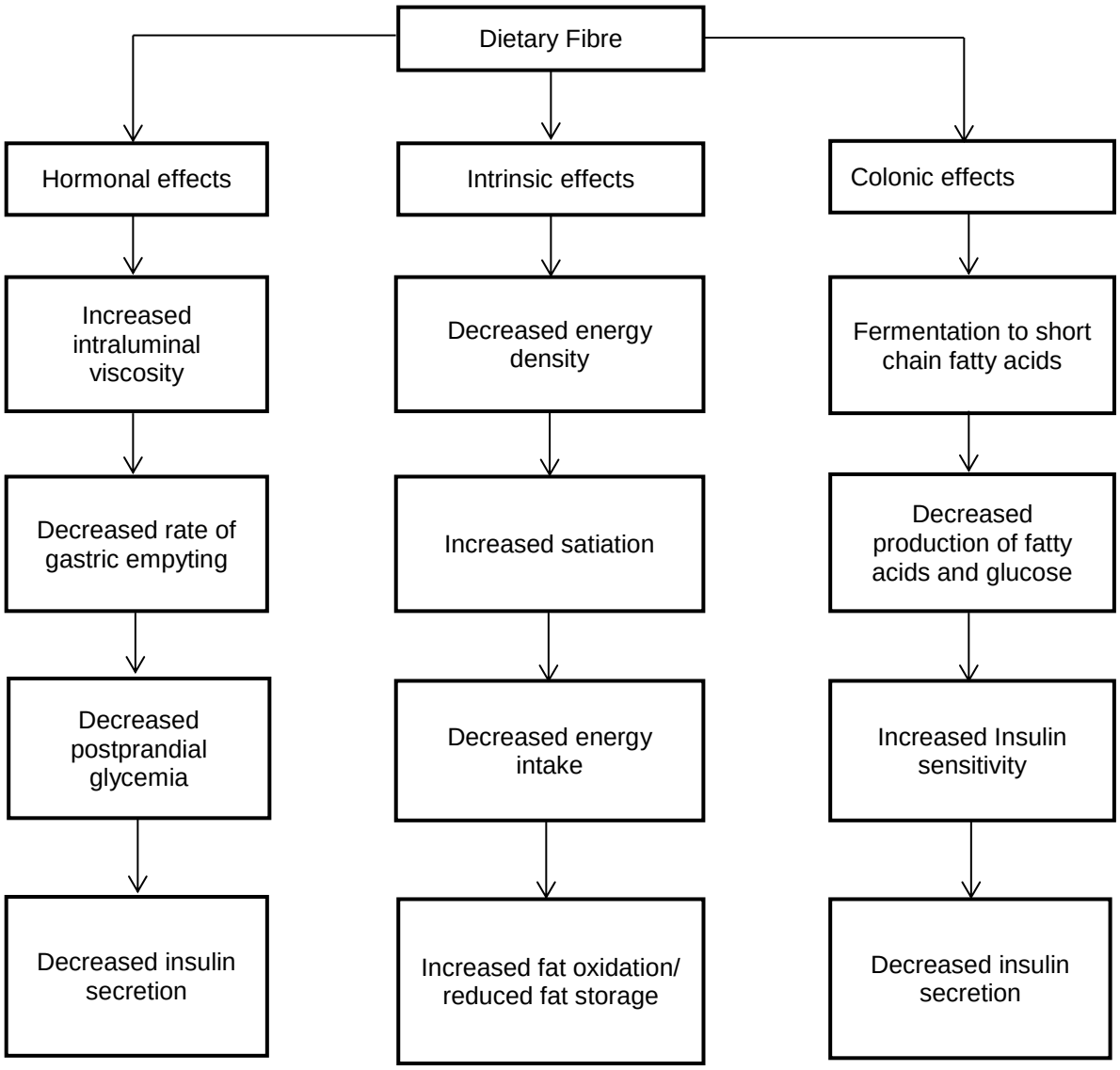

Figure 2.3 The various effects of dietary fibre in the human body. SCFA: short chain fatty acids. (Adapted from: Guillon & Champ, 2000; Chau & Huang, 2004; Khan *et al.*, 2007; Gropper *et al.*, 2009; Elleuch *et al.*, 2011; Huang *et al.*, 2011).

This amount is sufficient to fortify DF deficient foods. BGN DF has potential for use as a food ingredient, fibre supplement, fortifying agent and also in non-food applications (Fasoyiro *et al*, 2012).

2.2.4 Relevance of dietary fibre for the food industry

A higher amount of DF is found in less popular foods such as whole grains, legumes and fruits (Slavin, 2013). The food industry may use the properties of dietary fibre to their advantage by incorporating it in various food products (Tomaschunas *et al*., 2013). Table 2.4 shows the various industries that stand to benefit from the increased use of fibres. Dietary fibres can alter the consistency, rheological behaviour, texture and sensory properties of food products such as meat, baked goods, dairy, beverages, sauces, confectionery and fast foods (Guillon & Champ, 2000; Daou & Zhang, 2011). Furthermore, dietary fibres may be used as replacements to additives thus offering various products a 'clean label'.

Dietary fibre may be included in diets of groups that are prone to fibre deficiency such as dysphagic groups who might otherwise have low fibre intake (Daou & Zhang, 2011). In order for a particular fibre to be an acceptable food ingredient, it must among others, have a good shelf life, a high concentration in small quantities so as to maximise its use, a balanced composition of soluble and insoluble fibres as well as be compatible with food processing. Furthermore, the fibre must have a bland taste and not have anti-nutritional components, an offensive odour or negative colour and textural effects. The fibre must also have an adequate amount of associated bioactive compounds, be of reasonable cost and have a positive consumer image (Kunzek *et al.*, 2002; Figuerola *et al.*, 2005; O'Shea *et al*., 2012; Yangilar, 2013).

2.2.5 Physicochemical characteristics and recommended intakes of dietary fibre

The physical and chemical properties of fibre are of importance in predicting its functional behaviour mainly in food systems (Guillon & Champ, 2000). These properties include solubility, fat binding capacity, water holding capacity, density, swelling capacity, gel forming ability, mineral and organic molecule binding capacity, flavour, colour and rheological properties (Guillon & Champ, 2002; Biswas *et al*., 2011). All these characteristics play a major role in greatly affect the physical and chemical characteristics of the food systems they are used in.

Table 2.5 shows the recommended daily intakes (RDI) of DF by different organisations. Khan *et al*. (2007) reported that adults must consume up to 35 g of fibre per day with adequate fluid intake. Specifically the recommendations are broken down to 25 g/day for women younger than 50, 21 g/day for women older than 50, 38 g/day for men under 50 and 30 g/day for men over 50 years of age (Elleuch *et al*., 2011).

Table 2.4 Various properties of fibre that can be used in food products

Industry	Product	Property of fibre	Source
Processed meat	Sausages, polony	Fat replacer Emulsion stabiliser Water binding	Tomaschunas *et al*. (2013)
Bakery	Bread	Modify texture Increase volume Increase shelf life	Guillon & Champ (2000) Elleuch *et al*. (2011)
Dairy	Ice cream Yoghurts	Improve mouth feel Reduce syneresis	Elleuch *et al*. (2011)
Beverage	Juice	Bulking agent, nutritional additive	Daou & Zhang (2011)
Sauce	Sauces	Thickener	Guillon & Champ (2002)
Confectionery	Sweets	Reduce calorie content	Guillon & Champ (2002)
Fast food	Fried products	Reduce oil retention	Guillon & Champ (2002)
Extruded products	Pasta	Fortifying agent	Daou & Zhang (2011)

Table 2.5 Recommended daily intakes (RDI) for individuals 14 years or older

Organisation	RDI (g)
United States Food and Drug Administration (USFDA)	25
United States Department of Agriculture (USDA)	25
American Diabetes Association (ADA)	25 -35
American Dietetic Association	20 - 30
National Cancer Institute	20 - 30
Europe Union	20

(Adapted from: Borderias *et al*., 2005; Verma & Banerjee, 2010)

The increase in fibre consumption for promotion of health and prevention of diseases is a critical public health goal (Figuerola *et al*., 2005; Biswas *et al*., 2011; Cava *et al*., 2012).

2.3 Extraction and Fractionation of Dietary Fibre

The aim of separating fibres into their individual constituents is to isolate and quantify fractions of interest and eliminate unwanted compounds (Anon., 2010; Elleuch *et al*., 2011). While methods of quantifying fibre have been largely studied those of isolation and fractionation of fibres are limited (Elleuch *et al*., 2011). The extraction method and source of DF largely affect the properties and composition of the resultant fibres (Guillon & Champ, 2000; Huang *et al*., 2011). They also affect the behaviour of the fibres in the gastro-intestinal canal and in food applications (Figuerola *et al*., 2005). Cowpeas, lentils, chickpeas, pigeon peas, green peas and kidney beans are some legumes that have been employed in DF research (Dalgetty & Baik, 2003; Khan *et al*., 2007).

The basis of all the methods of extracting fibre is similar however the approach differs depending on desired end product, source of fibre and equipment used.

All the methods involve fractionation; this allows for the separation of constituents to obtain the desired concentrates and isolates (Anon., 2010). Some known methods of extracting dietary fibre include microbiological retting, chemical methods, enzymatic methods and mechanical separation (Bogracheva *et al*., 2001). Guillon & Champ (2002) stated that in the case of legumes, preparations obtained with hulls are richer in DF than those from dehulled seeds.

2.3.1 Dry processing methods

Dry processing methods involve the disintegration of seeds by milling and air classification into starch and protein fractions (Bogracheva *et al*., 2001). The flour produced during the milling process contains two distinct populations of particles which differ in size and density. To separate these two phases, a current of air is used, thus the origin of the name ‘air classification’. One phase consists of fine and lighter particles containing mostly starches and fibres while the other phase is coarse and relatively heavier containing mainly proteins and lipids (Bogracheva *et al*., 2001). To purify fractions, air classification is repeated on products, this however is a drawback as it reduces product recovery. Air classification is more efficient when used on products such as peas (*Pisum sativum*), faba bean (*Vicia faba*), baby lima bean (*Phaseolus lunatus*) and cowpea (*Vigna unguculata*), which have starch as their main storage material.

2.3.2 Wet processing methods

There are several wet processing methods discussed in literature. Wet milling methods all use water for fibre extraction but differ in the additional reagents and conditions. The wet

milling methods discussed in the following sections are the conventional wet milling, alkali wet milling, enzymatic wet processing and the modified wet milling method.

1. *Conventional wet milling method*

The traditional wet milling method involves the soaking (steeping) of raw materials in a solution of sulphurous acid (Ramirez *et al*., 2009). The co-products and starch obtained are then physically separated. Apart from being time and energy consuming, this process is also environmentally unfriendly due to the large amounts of sulphur dioxide (SO_2) required during the steeping step. When sulphurous acid reacts with water, SO_2 is produced (Ramirez *et al*., 2009). In the atmosphere, SO_2 can react with other polluting gases such as nitrogen dioxide (NO_2) forming acid rain. It is also associated with severe respiratory disorders and it particularly irritates asthmatic individuals (Ramirez *et al*., 2009). The traditional wet milling steeping process takes up to 36 h to complete. Conventional wet milling method would not be cost effective if applied to BGN fibre extraction.

2. *Alkali wet milling method*

The alkali wet milling involves soaking the plant material under study in NaOH (pH 13) at 85°C (AACC, 1987). The soaked material is then debranned, cracked and steeped in NaOH at 45°C then ground to a powder. The powder is then blended with the NaOH and the recovered slurry is degermed, ground, screened and washed through sieves. The residue is collected as fine fibre. The use of NaOH would negatively affect the colour and antioxidant properties of BGN fibres. Residues of NaOH are most likely to remain in the fibres hence making them less desirable for food uses. Furthermore whole seeds (hulls and endosperms) were desired in the extraction of fibre from BGN thus the debranning process would be a disadvantage.

3. *Enzymatic wet milling method*

To help curb the problems associated with SO_2, the AACC (1987) enzymatic wet milling is a common alternative. In this process, SO_2 is reduced to minimal levels and only imparts antimicrobial properties. The processing time for enzymatic wet milling saves energy and time. The enzymes commonly used are protease enzymes such as alcalase that solubilise and hydrolyse the gluten matrix (protein), heat stable α-amylase that gelatinises, hydrolyses and depolymerises starch and amyloglucosidase that disintegrates starch fragments to glucose (Ramirez *et al*., 2009). The unchanged non-starch polysaccharides are recovered by precipitation with ethanol, then washed and dried. Fibre is separated and recovered by eliminating loose starch and proteins by passing over a grit screen (Ramirez *et al*., 2009). Enzymes applied in fibre extraction are usually costly, hence the use of enzymatic wet milling in BGN fibre extraction would not have been cost effective.

4. *Modified wet milling method*

The traditional wet process is intended for food applications (Anon., 2010). Wet processing methods involve the use of water and produce products with a high purity that can be employed in applications such as scientific research (Bogracheva *et al*., 2001). Using these methods, the seeds are ground to very small particles to increase the surface area. The protein is then extracted at an alkaline pH and is subjected to acid precipitation (Bogracheva *et al*., 2001; Dalgetty & Baik, 2003; Anon., 2010). Sodium hydroxide is commonly employed to provide the alkaline pH.

To isolate IDF, a difference in swelling properties of the fractions is applied (Dalgetty & Baik, 2003). At room temperature fibre has a high swelling capacity whilst the swelling of starch is very restricted. Such differences in swelling capability gives rise to different sizes. The insoluble extract fraction is dispersed in a large amount of water and screened through a series of sieves with pore diameters ranging from 30 - 300 µm (Bogracheva *et al*., 2001). The supernatant is mainly a dispersion of starch granules and the residue is mainly fibre (Anon., 2010).

In industry, the fibres are dried by spread driers specially constructed for that purpose (Bogracheva *et al*., 2001). For scientific research purposes, freeze drying of the different fractions is more appropriate (Bogracheva *et al*., 2001). The wet milling method to be used in this research uses much less water than other wet milling methods and uses minimal chemicals (Dalgetty & Baik, 2003).

2.3.3 Physical and microbial methods

Physical methods of fibre extraction preserve the structure of the fibres and avoid significant damage to the polymer chain. As a result, the extracted fibres tend to have a high cation exchange capacity as the side chain group remains almost intact (Yangilar, 2013). Not much research has been carried out on these methods.

Microbial methods involve the fermentation of fibre using microorganisms and enzymes. Most of the known methods are very specific and precise. Enzymes of high purity are often used to selectively remove oligosaccharides and polysaccharides such as galactans, fructans, mannans and arabinans (Rodriguez *et al.*, 2006). Some of the advantages of microbial isolation are that the structure of the fibres remains undistorted and significant hemicelluloses and soluble fibres are not lost. In addition, the methods have high selectivity and are easy to handle. On the negative side, it is suspected that microbial fermentation produces toxic substances, hence making the extracted fibres unsuitable for use in food applications (Yangilar, 2013).

2.3.4 Gravimetric methods

1. *Nonenzymatic-gravimetric methods*

Nonenzymatic-gravimetric methods are one of the earliest methods developed for fibre extraction. The methods include hydrolytic or oxidative chemical decomposition leaving behind crude fibre. These methods can be placed into two categories (Elleuch *et al.*, 2011). The first category includes acid-detergent and neutral detergent extractions. The acid-detergent procedure isolates crude fibre as the sum of cellulose, lignin and acid insoluble hemicelluloses; as a result, most of the fibre components are lost. The neutral detergent procedure isolates cellulose, lignin, and neutral detergent-insoluble hemicelluloses (Elleuch *et al.*, 2011). It is however unsuitable for plants that are high in soluble fibre such as BGN. The second category makes use of protein and starch-digesting enzymes and is discussed in detail under the enzymatic-gravimetric methods.

2. *Enzymatic-gravimetric methods*

The enzymatic-gravimetric method was developed by Prosky *et al*. (1988) based on the work of Asp (1978). This method makes use of enzymatic removal of starch and proteins followed by the precipitation of the soluble fibre concentrate using ethanol (Dhingra *et al.,* 2012). The gravimetric method starts with the use of alkalis and acids to determine crude fibre in plant samples and was later modified by the AOAC to include animal feed. This method was further modified to include the use of enzymes to remove starch and solubilize the protein fraction (AOAC, 2000). The modified method involves the removal of fat if present above 10%.

The enzymatic-gravimetric method has evolved to make use of 4-morpholine-ethanesulfonic acid-TRIS (MES-TRIS) buffer in place of the original phosphate buffer, thus saving time and energy associated with the continuous pH adjustments (Elleuch *et al.*, 2011). A modern enzymatic-gravimetric method involves suspending samples in acetate buffer at pH 5 then digesting with heat stable α-amylase at temperatures between 95 - 100°C for 30 min – 1 h to digest starch (Diedericks, 2014). The samples are further incubated at 60°C with protease followed by digestion with amyloglucosidase at 60°C to breakdown starch fragments to glucose monomers (Salehifar & Fadaei, 2011; Daou & Zhang, 2011). Soluble fibres are then precipitated with ethanol and total fibre is recovered by centrifugation with ethanol and acetone and left to dry at room temperature (Daou & Zhang, 2011; Graham *et al.*, 1988).

Fibre was extracted from coconut residue using cold, slightly alkaline water followed by centrifugation at room temperature (Verma & Banerjee, 2010). The residues were extracted with EDTA (Fudar, 1977). The resultant residues were rinsed with ethanol and deionized water, then treated with enzymes as discussed in the enzymatic-gravimetric method (Verma & Banerjee, 2010). The enzymatic-gravimetric method was applied in

extracting fibre from defatted rice bran; however, alcalase was used as the protein-degrading enzyme (Daou & Zhang, 2011). The use of pepsin and pancreatin for the digestion of protein and starch is suggested as these enzymes would mimic the alimentary digestive enzymes. Enzymatic-gravimetric methods are tedious and costly hence would not be cost effective if applied to BGN fibre extraction.

2.3.5 Enzymatic-chemical methods

The enzymatic-chemical method was first outlined by Southgate (1969) and developed by Englyst *et al*. (1994). These methods involve the enzymatic digestion of nonfibre fractions coupled with chemical removal of the fractions. Specifically, the method involves the enzymatic removal of starch and the use of ethanol to isolate the soluble fibre concentrate from products of starch hydrolysis and low molecular weight sugars. Ethanol is commonly employed in these methods to precipitate solubilised fibre components as in the enzymatic-gravimetric methods (Diedericks, 2014). A crucial step was the removal of all starch to avoid overestimation of total dietary fibre extracted. This was accomplished by use of dimethyl sulfoxide (Elleuch *et al.*, 2011).

The initial step of the AACC method involves digestion using H_2SO_4 followed by filtration using water and NaOH. The samples are then washed with H_2SO_4 and ethanol and dried in a muffle oven. The alcoholic fibre extraction method involves boiling material in water for 3 h and extraction using 95% ethanol, followed by agitation overnight and filtration through a nylon bag using a hydraulic press (Prakongpan *et al.*, 2002). The fibre is then recovered by air-drying the residue for 6 h. Fibre can be subjected to alkali digestion, strained through a cheese-cloth then oven dried overnight (Prakongpan *et al.*, 2002). It is recommended to reduce the concentration of ethanol that is used in the precipitation of soluble fibre from 76% to between 41% and 56% to reduce cost and also reduce environmental chemical contamination (Prakongpan *et al.*, 2002). The use of chemicals negatively affects the antioxidants composition of fibres hence reducing their desirable characteristics. Chemical residues may also remain in the fibres making them less suitable for human consumption.

2.3.6 Extraction of individual constituents of fibre

Insoluble pectic substances can be extracted from plant material using heated ammonium oxalate solution followed by filtration, washing with ethanol and distilled water. Hemicelluloses can be extracted from depectinated fibre by centrifugation of the samples with 5% KOH. The residue termed lignocellulose is further centrifuged with 50% acetic acid at pH 5.0 – 5.5. The residue is dried as hemicellulose. Cellulose can be extracted using $KMNO_4$ mixed with lignin buffer in a ratio 2:1 followed by addition of demineralized solution. Lignin can be extracted using H_2SO_4 at refrigeration temperature. Cold distilled water is then

added and after precipitation acid is washed off the residue with warm distilled water. The crude lignin can then be air dried (Verma & Banerjee, 2010).

2.3.7 Optimisation of fibre extraction methods

A method of extracting soluble dietary fibre from wheat, rye, barley, oats, potatoes, carrots, lettuce and peas was optimised by Graham *et al*. (1988). The study compared four extraction conditions: 1) acetate buffer at pH 5, 96°C for 1 h followed by starch degradation at 60°C for 4 h, 2) water extraction at 38°C for 2 h, 3) HCl/KCl buffer at pH 1.5 at 38°C for 2 h, 4) pretreatment with absolute ethanol at 96°C for 1 h followed by water extraction at 38°C for 2 h. High temperature extraction gave the highest extraction yield while acidic extraction gave the least. Carrots yielded the highest total dietary fibre (TDF) and potatoes yielded the least. The researchers concluded that extraction conditions affect the yield and the composition of the resultant fibres.

The extraction of dietary fibre from date seeds was optimised. The seeds were dried at 50°C for 2 days, ground and then fibre was extracted using water on some samples and acetone on others (Al-Farsi & Lee, 2008). After stirring for 1 h at 40°C, centrifugation and filtration, butanone and butanol were employed for purification of the fibres. The residue was dried at 60°C and ground as dietary fibre concentrate. The researchers concluded that the use of water extraction followed by purification using butanol gives the highest yield as compared to water-butanone, acetone-butanone and acetone-butanol. The researchers chose butanol and butanone for purification because of their low toxicity levels, economy due to low evaporation temperature, and high affinity to dissolve compounds with hydroxyl groups. The yield of total dietary fibre from the date seeds was 93.5% (water-butanol), 91.2% (water-butanone), 88.8% (acetone-butanol) and 81.9% (acetone-butanone). Acetone was found to extract a higher amount of phenolics with the fibre because of its high extraction efficiency.

The extraction of soluble dietary fibre from defatted rice bran was optimised using response surface methodology (Wan *et al.,* 2002). The independent variables they investigated were the ratio of $Ca(OH)_2$ solution to defatted rice bran, concentration of $Ca(OH)_2$, and extraction temperature, with optimal values of 29.75:1 (mL/g), 3%, and 84°C (1 h), respectively. The method involved digesting defatted rice bran with α-amylase at 90°C and pH 6.9 for 15 min. After digestion the mixture was filtered, washed with deionised water and mixed with $Ca(OH)_2$ for 4 h. The alkali was then neutralised with acetic acid, centrifuged, dialysed against deionised water and precipitated with 80% ethanol. The yield of fibre was 7.86 g/100 g.

Fibre extraction from Maixiansan was optimised using response surface methodology (Lv *et al.,* 2010). The procedure involved soaking Maixiansan in boiled water, digestion of starch with α-amylase until the enzyme was inactivated, washing, centrifugation and drying in

a rotary evaporator. After optimisation, the highest fibre yield of 57.1% was obtained using an enzyme concentration of 0.4%, 45 min enzymolysis time and 4% NaOH content.

Soluble fibre was extracted from defatted rice bran using 1) NaOH pH 14, 2) CaOH pH 12, 3) $NaCO_3$ pH 11, 4) acetic acid pH 3 and 5) HCl pH 0.5 (Fadaei & Salehifar, 2012). All the treatments involved the pre-digestion of starch using glucoamylase, blending sample with the appropriate reagent followed by shaking at 60°C for 4 h, centrifugation, and neutralising with acetic acid. Some samples were treated with trichloroacetic acid. The samples then underwent dialysis under tap water for 3 days, then soaked in 95% ethanol overnight, centrifuged then re-dissolved in water and freeze dried. The yield of fibre was 8, 5, 2, and 4% for each treatment, respectively. Trichloroacetic acid was applied for protein removal; however, it was noted that it was inefficient as there was no significance difference in the protein content of treated and untreated samples. It was observed that even though extraction with NaOH gave the highest yield, it was inappropriate for food applications as it imparted a strong brown colour to the fibres. Treatment with CaOH would be more appropriate because it gave the least discoloration, had a desirable composition of fibres, and a satisfactory yield. In addition, the fibres treated with CaOH retained their hypocholesterolemic activity.

2.3.8 Comparison of fibre extraction methods

Dry processing methods are suitable for plants that have starch as their main storage material. These methods require repeated classification to purify fractions and this is a drawback as it reduces product recovery (Bogracheva *et al.*, 2001). The conventional wet milling process makes use of large amounts of sulphur dioxide (SO_2) during the steeping step. SO_2 is environmentally unfriendly as it reacts with other polluting gases such as nitrogen dioxide (NO_2) in the atmosphere forming acid rain. It is also associated with severe respiratory disorders and it particularly irritates asthmatic individuals (Ramirez *et al.*, 2009). The conventional wet milling steeping process is very time consuming, taking up to 36 h to complete. In the conventional wet milling method, steeping and evaporation of steep water is estimated to use up to 21% of the total capital and energy, hence making it very costly (Eckhoff *et al.*, 1999).

The alkali wet milling method is equally tedious and time consuming as the conventional wet milling method. The process however produces a relatively environmentally acceptable stream of waste water (Eckhoff *et al.*, 1999). The enzymatic wet milling method was developed to overcome the problems associated with the conventional wet milling process. In this method, SO_2 is reduced to minimal levels to impart antimicrobial properties and the processing time is also reduced (Ramirez *et al.*, 2009; Prosky *et al*., 1988).

The modified wet milling method involves the use of water and produces products with a high purity that can be employed in various applications including scientific research (Bogracheva *et al.*, 2001). The modified wet milling method uses much less water than the traditional wet milling method and does not require the use of any chemical (Dalgetty & Baik, 2003).

Enzymatic-gravimetric methods extract a group of polysaccharides, lignin, some resistant starch, waxes, Maillard reaction products and phenolic compounds. They give a higher yield of fibre of almost two-fold compared to enzymatic-chemical methods (Salehifar & Fadaei, 2011; Gordon &Okuma, 2002). They are also quick, easier to carry out and do not overestimate fibre (Dhingra *et al.*, 2012). The limitation of these methods is that some insoluble polysaccharides, lignin and all soluble polysaccharides are lost. The residue obtained also contains some nitrogenous material and oligosaccharides and some resistant starch are not quantified (Elleuch *et al.*, 2011; Gordon & Okuma, 2002). Dialysis, which is employed in the purification of soluble fibre, is costly.

The enzymatic-chemical method is faster and easier to perform relative to nonenzymatic-gravimetric methods (Caprita & Caprita, 2011). However, it is environmentally unfriendly due to the use of various chemicals, which if not correctly handled or disposed of pose a threat to the environment (Al-Farsi & Lee, 2008). These methods are also tedious, time consuming and chemicals are also of concern due to the possibility of solvent residues in the product (Al-Farsi & Lee, 2008; Dhingra *et al.*, 2012).

Nonenzymatic-gravimetric methods also make use of chemicals and isolate cellulose, lignin and acid insoluble hemicelluloses. The use of chemicals improves the removal of starch and proteins, thus providing a fibre of high purity. However, chemical methods have poor selectivity and the extraction conditions are difficult to control, thus limiting their use (Yangilar, 2013). In addition, alkaline solutions dissolve hemicelluloses and some soluble fibres, therefore rendering them unavailable in the extracted fibre (Daou & Zhang, 2011).

An ideal method of extraction ought to be environmentally friendly, safe, easy to perform, and cost effective. Chemicals, enzymes and equipments used in many of the methods tend to be very costly. The wet milling method makes use of water and minimal chemicals and thus, is concluded to be one of the most cost effective methods of fibre extraction. Table 2.6 gives the advantages and disadvantages of the discussed fibre extraction methods. Knowledge of the advantages and limitations of extraction methods is of importance when selecting a method for extracting a particular type of fibre from a certain plant. A method that is less costly and uses minimal chemicals is considered preferable. With advances in technology and an increase in the knowledge pool, more effective, robust and reproducible fibre extraction methods have been developed (Bach Knudsen, 2001). However, the work done on fibre extraction methods is still very limited and more research is required.

Table 2.6 Advantages and limitations of fibre extraction methods

Method	Advantages	Limitations	References
Dry processing	Reduced water and energy consumption No reagents used	Only for plants with starch as main storage Low yield	(AACC, 2001; Ramirez *et al.*, 2009)
Conventional wet milling	Appreciable amount of fibre obtained	Large amounts of SO_2 Time consuming Very costly	(Salehifar & Fadaei, 2011)
Alkali wet milling	Less waste water produced	Tedious Time consuming	(AACC, 1987)
Enzymatic wet milling	SO_2 reduced to a minimum Processing time reduced	Possible SO_2 residues in product	(Salehifar & Fadaei, 2011)
Modified wet milling	High purity products Much less water used No chemicals	Waste water	(Ojimelukwe, 2009)
Enzymatic-gravimetric	Higher yield than enzymatic-chemical Quick and easy to carry out	Some insoluble fibre, lignin and all soluble fibre are lost Residues contain nitrogenous material	(Gordon & Okuma, 2002)
Enzymatic-chemical	Fast and easy to perform compared to enzymatic-gravimetric	Chemical residues in products Time consuming	(Bogracheva *et al.*, 2001) (Dhingra *et al.*, 2012)
Non-enzymatic-gravimetric	High purity fibres	Poor selectivity Extractions conditions difficult to control	(Mwaikambo, 2006)
Physical	Structure of the fibres preserved	Unreliable	(Mwaikambo, 2006)
Microbial	Structure of fibres maintained High selectivity Easy to handle	Toxic substances produced	(Mwaikambo, 2006)

2.3.9 Effects of extraction conditions on the characteristics of fibres

Different extraction conditions affect the characteristics of the resultant fibres. Enzymatic digestion and alkaline extraction partially delignifies lignocelluloses (Elleuch *et al.*, 2011). This results in improved fibre functionality in food products. Enzymes also modify the soluble fibre to insoluble fibre ratio; for example, xylanase increases the level of soluble dietary fibres when applied to cell walls. Mechanical applications such as grinding prior to extraction disrupt the fibre network exposing hydroxyl groups, thus increasing the water holding capacity of fibre and increase the surface area, hence increasing the fibre yield after extraction. However, excessive grinding results in reduced water holding capacity due to extensively disrupted fibre. Mechanical applications also affect the particle size of the resultant fibres. The smaller the particle size the higher the fat binding capacity.

Physical extraction methods result in an increase in total dietary fibre and an overall decrease in lignin content. In addition, the process increases the soluble to insoluble fibre ratio and also increases the water holding capacity (Dhingra *et al.*, 2012). Soaking, a common stage in the preparation of plant material prior to extraction, modifies the composition and availability of fibres. Heat changes the soluble to insoluble fibre ratio and alters the total dietary fibre content. The increase in fibre content is attributed to the formation of fibre-protein complexes that are heat resistant and are collected as fibre.

2.4 Prebiotics and Probiotics – Human Diet

FAO (2001) defines a prebiotic as an ingredient which is fermented by intestinal microflora and promotes the growth and activity of the microflora as well as bestows health benefits upon the host. The concept of prebiotics has received a lot of scientific and industrial interest since its introduction (Cummins *et al*., 2001; FAO, 2001; Gibson *et al*., 2004). Slavin (2013) reviewed the health outcomes of DF and prebiotics in the human body and showed the many beneficial effects these have on the body. For a food component to be classified as a prebiotic, it must meet three criteria, which are:

1. Should not be absorbed in the intestines and should not be hydrolysable by gastric acid or mammalian enzymes.
2. Should be fermentable by intestinal microflora.
3. Should selectively stimulate the growth and activity of intestinal microflora that contribute to the health and well-being of the host (FAO, 2001; Probert *et al.*, 2004; Gibson *et al*., 2004; Roberfroid, 2007).

Although all prebiotics are fibres, the vice versa is untrue (Slavin, 2013). Hence, the effect of DF extracted from different sources on probiotics needs to be studied to establish whether the particular fibre is fermentable and can selectively encourage the proliferation and activity of the bacteria. The prebiotic effect of a substrate can be determined either *in vivo* or *in vitro* (Rycroft *et al*., 2001; Manderson *et al*., 2005; Hur *et al*., 2011).

Fotiadis *et al*. (2008) defined a probiotic as a live microorganism which when ingested in certain numbers, provides health benefits beyond inherent general nutrition. In this research, *Bifidobacterium* spp. have been selected as the probiotics of interest on which to study the prebiotic effects of BGN DFs. According to Picard *et al*. (2005) *Bifidobacterium* constitute up to 25% of faecal bacteria in adults and up to 80% in infants. *Bifidobacterium* is commonly found in breast-fed infants because the human milk consists of human oligosaccharides that support the growth of *Bifidobacterium* (Martin *et al*., 2009). The fermentation of BGN DF by the *Bifidobacterium* spp. would open room for use in foodstuffs including baby foods.

2.4.1 Prebiotic nature of dietary fibre

The prebiotic properties of DFs can be harnessed and utilised in developing a wide range of products. Dietary fibre is categorized as a neutraceutical which has been shown to have the potential to impart health benefits if consumed in adequate quantities (Verma & Banerjee, 2010). Most constituents of DF are fermented by anaerobic bacteria in the large intestines, producing short chain fatty acids (SCFAs) such as butyric acid, propionic acid and acetic acid as well as gases such as hydrogen, carbon dioxide and methane (Khan *et al*., 2007). These SCFAs increase lipid metabolism and play a role in the absorption of toxic substances and excess bile acids hence aiding their elimination from the body. In addition, SCFAs stimulate cell division and regulate apoptosis thus promoting the health of the colon (Verma & Banerjee, 2010). In this manner, DF plays a role in preventing health complications such as colon cancer. Although a range of natural fibres have been previously studied by other researchers, the use of BGN fibres as a natural source of fibre and as a prebiotic has not been reported. The prebiotic properties of BGN fibres would be of use in many food products and prebiotic supplements. To quantify SCFAs produced in the fermentation of substrates, high performance liquid chromatography (HPLC) or gas chromatography (GC) can be used (Martin-Pelaez, 2008; Vigsnæs *et al*., 2011; Gietl *et al*., 2012).

2.4.2 *In vivo* and *in vitro* assessment of prebiotics

To assess the prebiotic characteristics of a substrate, *In vivo* or *In vitro* studies are employed. *In vivo* studies refer to procedures conducted within a whole, living organism. These are carried out through appropriate nutritional intervention trials in study subjects such as humans or animals using validated methods to obtain comprehensive scientific data (Roberfroid, 2007). *In vitro* studies are techniques of performing given procedures in controlled environments outside a living organism. One drawback of these techniques is that they fail to replicate the exact conditions of an organism.

In vitro studies applied in prebiotic studies make use of batch or continuous culture fermentation systems (Manderson *et al*., 2005; Sannasiddappa *et al*., 2011). Culture vessels are inoculated with pure cultures of selected species of bacteria and the carbohydrate to be

studied (Manderson *et al*., 2005). Multichamber continuous-culture systems have been developed to mimic the human digestive system, including portraying some steps involved in digestion and fermentation that would occur *in vivo* (Hobden *et al.*, 2013). Batch culture fermentation systems on the other hand, are divided into two classes, the non-stirred, non-pH controlled *in vitro* fermentations and the stirred, pH controlled *in vitro* fermentations. In non-stirred, non-pH controlled *in vitro* fermentations, anaerobic conditions are maintained by use of anaerobic jars and anaerobic gas generating kits. However, it is known that SCFAs accumulate in static batch cultures there by inhibiting the growth of microorganisms by reducing pH. To prevent this from occurring, the buffer concentration is increased such that pH does not drop below 5 (Gietl *et al.*, 2012). In stirred, pH controlled *in vitro* fermentations, anaerobic conditions are maintained by continuously sparging oxygen-free nitrogen gas into the medium and pH is monitored over the fermentation period (Martin-Pelaez, 2008). Batch fermentations are commonly run for 24 h to simulate the incubation time in the large intestines of monogastric animals (Martin-Pelaez, 2008). In the current study, static batch fermentation systems were employed and the fermentations were run for 24 h.

2.5 Emulsions

An emulsion is a colloid that consists of two immiscible liquids, usually oil and water, with one of the liquids dispersed in the other (McClements & Weiss, 2005). Emulsions consist of two phases, a dispersed and a continuous phase. The dispersed phase consists of the particles that make up the droplets and the continuous phase is the surrounding liquid in which the droplets are dispersed in (Robins *et al.*, 2002). The diameters of droplets in most foods usually ranges from 0.1 – 100 µm (Stern *et al*., 2001). A system that with oil droplets dispersed in an aqueous phase is known as an oil-in-water (O/W) emulsion and the vice versa is called water-in-oil (W/O) emulsion (Garti & Benichou, 2004; McClements & Weiss, 2005). Pure oil and water emulsions are possible, but the two liquids rapidly separate to a layer of oil on top of water (Yang & Lai, 2003). This happens because of the thermodynamically unfavourable contact between oil and water molecules (Kerkhofs *et al.*, 2011; Zhang, 2011). Although emulsions are generally thermodynamically unstable, they can be kinetically stable for a long time in the presence of emulsifiers (Zhang, 2011). Emulsions can be categorised according to the relative spatial distribution of the oil and aqueous phase. Emulsions can be classified according to the nature of the emulsifying agent or the arrangement of the system as shown in Table 2.7. Examples of emulsions include oil-in-water, water-in-oil, macro-emulsions, micro-emulsions, bilayer droplets, multiple emulsions, mixed emulsions, pickering emulsions and glassy emulsions. In the present study, orange oil-in-water emulsions stabilised with BGN soluble dietary fibres were studied.

Table 2.7 Classification of different types of emulsion

Nature of emulsifier	System organisation	Source
Non-ionic surfactants	Oil-in-water, water-in-oil	Ghosh & Rousseau (2011)
Ionic surfactants	Macro-emulsions	Adeyi (2014)
Mixture of surfactants	Micro-emulsions	Garti & Benichou, 2004
Non-ionic polymers	Bilayer droplets	McClements & Weiss (2005)
Polyelectrolytes	Multiple emulsions	Kumar *et al.* (2012)
Mixture of polymers and surfactants	Mixed emulsions	Adeyi (2014)
Solid particles	Pickering emulsion	Tadros (2013)
Liquid crystalline phases	Glassy emulsion	Tadros (2013)

2.5.1 Emulsion applications in food systems

Emulsions are very common in the food industry with O/W emulsions being more popular that W/O emulsions. Examples of food emulsions are mayonnaise which is an O/W emulsion stabilised by the egg york phospholipid, lecithin (Adeyi, 2014). Homogenised milk is also an O/W emulsion stabilised by milk proteins. Salad dressings and vinaigrettes are O/W emulsions that are sometimes prepared without an emulsifier giving a desirable separation which is characteristic of the products. Butter is a W/O emulsion consisting of water dispersed in butterfat (Garti & Benichou, 2004).

Beverage emulsions are defined as O/W emulsions that can be classified as flavour emulsions or as cloud emulsions (Reiner *et al*., 2010; Gharibzahedi *et al.*, 2012; Cheong *et al*., 2014). Examples of beverage emulsions are fruit drinks, sodas and punches (Mirhosseini *et al*., 2008). In these emulsions, the oil phase is the flavour oil such as terpenes and the aqueous phase is a solution of highly functional hydrocolloids such as gums (Gharibzahedi *et al.*, 2012). The instability of emulsions is a challenge especially to beverage manufacturers as beverages are very dilute with as little as 20 mg/L oil phase present in the final product (Reiner *et al*., 2010). Beverage emulsions are prepared as concentrates that are diluted into finished products (Chanamai & McClemments, 2001). Beverage emulsions are expected to be stable, both as concentrates and as dilute, for a period of at least 6 months (Mirhosseini *et al*., 2008). These emulsions are usually stabilised by polysaccharides such as hydrocolloids or modified starches (Chanamai & McClemments, 2001). In the current study, BGN soluble dietary fibres were applied as stabilisers in orange oil beverage emulsions.

2.5.2 Emulsion stability

The stability of emulsions is their ability to maintain their properties; that is the ability of the phases of the emulsion to remain mixed together. The stability of an emulsion determines its shelf-life of food emulsion products (Anon., 2011b). Various factors affect emulsion stability and these include: distribution of particle sizes within the emulsion, differences in densities between the droplets and the medium, phase inversion, coalescence, flocculation, Ostwald ripening, creaming, oxidation and degradation (Zhang, 2011).

Flocculation is when the droplets in an emulsion are attracted to each other and form flocs without the rupture of the stabilising layer at the interface (Adeyi, 2014). Droplet flocculation can be due to forces such as gravity, centrifugation, Brownian forces as well as when the repulsive energy is less than van der Waals energy (Adeyi, 2014). This is disadvantageous because flocculation promotes creaming and reduces clouding due to larger particle sizes as well as promotes coalescence due to droplets being brought closer together (Chanamai & McClemments, 2001).

Creaming is when emulsion droplets merge together forming bigger droplets or when the droplets rise to the surface of the emulsion due to buoyancy. This is usually a result of gravitational force when the density of the dispersed phase is less that the density of the medium (Zhang, 2011; Tadros, 2013). Creaming is usually a precursor of coalescence and is followed by phase separation (Anon., 2011b). Creaming occurs gradually and at different rates depending on the emulsion. As it progresses, the emulsion eventually loses its stability. Creaming can be reduced by adding a thickener to the continuous phase (Adeyi, 2014).

Ostwald ripening is a phenomenon whereby larger droplets expand at the expense of smaller ones and is largely affected by the solubility of the dispersed phase in the continuous phase (Zhang, 2011).

2.5.3 Emulsifiers and stabilisers

An emulsifier is a surface active molecule that adsorbs to the surface of freshly formed droplets of an oil-water interface during homogenisation, forming a protective membrane that prevents the droplets from aggregating (Stern *et al.*, 2001). They are amphiphilic in nature possessing both hydrophilic portions that align with the aqueous phase and hydrophobic portions that align with the lipid phase (Stern *et al*., 2001; Zhang, 2011). In this manner they act as surface-modifying substances at the interface between each droplet and the continuous phase (Robins *et al.*, 2002). Some emulsifiers are naturally present in commercial emulsifying ingredients like lecithin in egg yolk and some are synthetic (Dickinson, 2008).

Stabilisers are a group of additives that are capable of stabilising emulsions. Stabilisers may be classified either as emulsifiers or texture modifiers depending on their mode of action (Yang & Lai, 2003). Texture modifiers can also be used to stabilise emulsions, as they have the ability to enhance emulsion stability by retarding the movement of the droplets (Kerkhofs *et al.*, 2011). Texture modifiers can be placed into two groups, thickening agents and gelling agents, depending on their mode of operation and rheological behaviour (Stern *et al*., 2001). Thickening agents increase the viscosity of emulsions, whereas gelling agents form a gel in the continuous phase of emulsions.

2.6 Rheological Properties of Food Systems

Rheology is defined as the science that is concerned with the deformation and flow of matter under stress (Sivam *et al*., 2010*;* Sadiku-Agboola *et al.*, 2011). Of particular importance in food system is viscosity which is defined as the resistance of a fluid to flow caused by internal friction due to molecular attraction. It is a measure of the resistance of a fluid to deformation either by shear or extensional stress (Sahin & Sumnu, 2006). Shear stress can be explained as the state of stress where the stress is tangential or parallel to the face of a

material. Materials can be described as Newtonian or non-Newtonian depending on their flow behaviour in response to an applied shear stress or shear rate (Partal & M[a] Franco, 2006). Figure 2.4 shows the classification of rheological systems.

The Newtonian system is the “ideal” rheological system where the shear stress has a direct relation to the shear rate and the viscosity is constant and reversible under conditions of shear (Chhabra & Richardson, 2008). Newtonian behaviour is shown by all gases, liquids such as water, glycerol and chloroform, true solutions such as sugar syrups, solutions of low molecular weight as well as dilute colloidal dispersions.

A non-Newtonian system is one where the shear stress is not directly related to the shear rate and the viscosity varies with shear rate or stress and could also be dependent on time and reversible under conditions of shear (Chhabra & Richardson, 2008). Most liquid systems display this behaviour and exhibit shear-rate dependent viscosity termed apparent viscosity (Partal & M[a] Franco, 2006). If this viscosity increases with increasing shear rate the material is said to be shear-thickening and if it decreases the material is shear-thinning (Partal & M[a] Franco, 2006). Non-Newtonian behaviour is most frequently pronounced at intermediate shear rates. At extraordinarily high or low shear rates, many non-Newtonian systems approach Newtonian behaviour.

The rheological behaviour and flow properties of food products can be related to the mouth-feel or textural properties of the foodstuff and are highly dependent on the type and concentration of the ingredients within the foodstuff (Gallegos *et al.,* 2004; Fischer & Windhab, 2011). Several hydrocolloids have been researched for use in stabilising food emulsions by increasing the viscosity. These include gum Arabic, larch, tragacanth, guar, xanthan, starch, galactomannans, locust bean gum, BGN flour and other polysaccharides (Chanamai & McClements, 2000; Yadav *et al.*, 2007; Acton, 2012; Gharibzahedi *et al.*, 2012; Cheong *et al*., 2014; Adeyi, 2014). Different stabilisers behave differently in food systems due to factors such as degree of particle depression, molecular weight, molecular shape and strength of intramolecular bonds (Adeyi, 2014).

2.7 Summary and Conclusions

Bambara groundnut is a promising legume, nutritionally rich and considered a complete food. Notably, is the high content of dietary fibre in this legume. The inclusion of BGN dietary fibre in food products could play a major role in marketing BGN, consequently increasing its market value. This would further result in this legume playing a role in increasing food security.

.

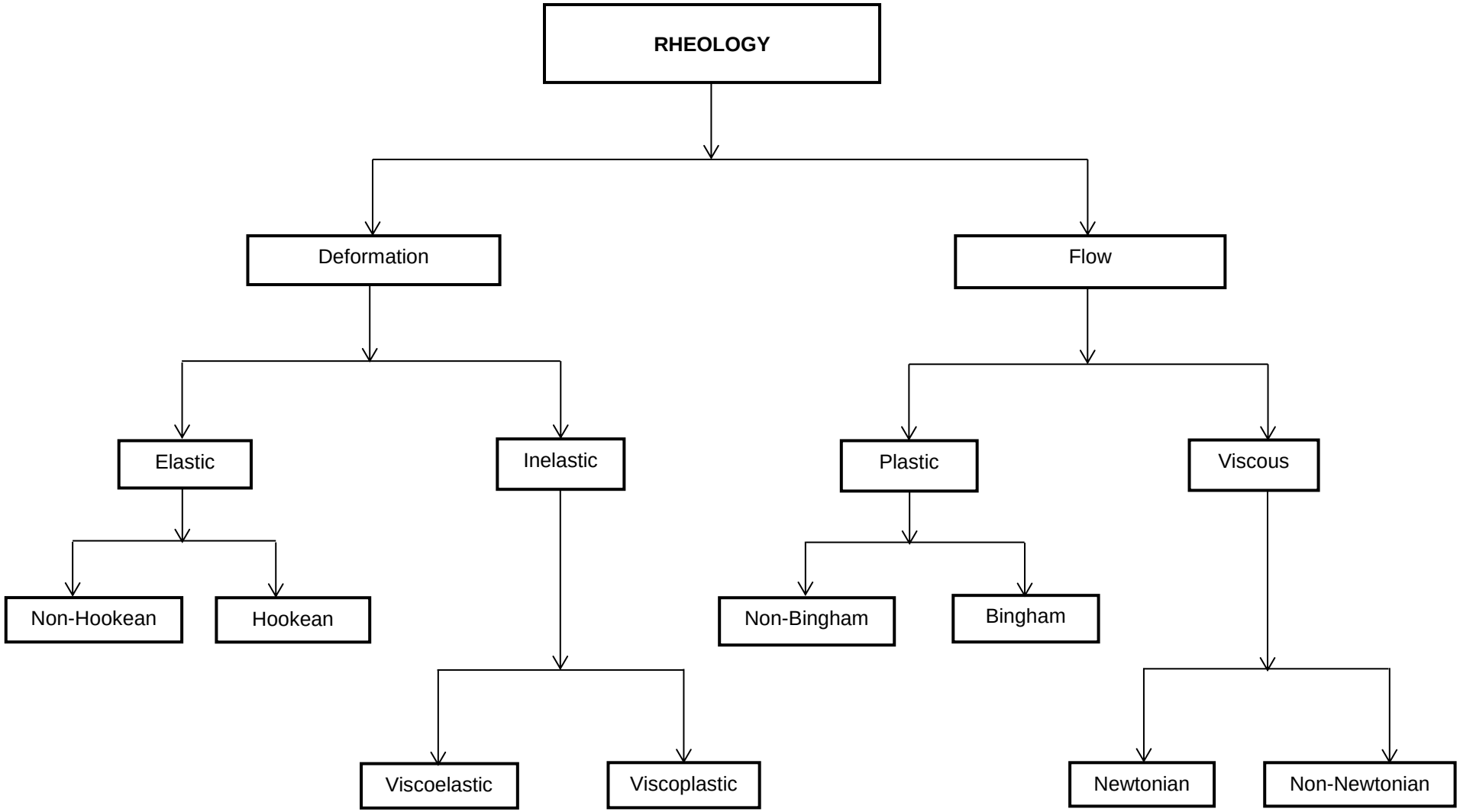

Figure 2.4 Classification of rheological systems. (Adapted from: Sahin & Sumnu, 2006).

References

AACC American Association of Cereal Chemists (1987). (edited by A.W. Stanley & P.E. Ramstad. St. Paul Minnesota: Corn Chemistry and Technology.

AACC American Association of Cereal Chemists (2001). The definition of dietary fibre. AACC Report. *Cereal Food World*, **46**, 112 - 126.

Acton, A. (2012) Beverage emulsions as functions of main emulsion components. In: *Issues in Food production, Processing and Preparation*. Atlanta: Scholarly Editions.

Adeyi, O. (2014). Effect of Bambara Groundnut flour on the stability and rheological properties of oil-in-water emulsion. DTech Thesis, Cape Peninsula University of Technology.

Afoakwe, E. O., Budu, A. S. & Merson, A. B. (2007). Response surface methodology for studying the effect of processing conditions on some nutritional and textural properties of Bambara groundnut (*Voandzei subterranean*) during canning. *International Journal of Food Science and Nutrition*, **58**(4), 270 - 281.

Ajayi, F. A. & Lale, N.E.S. (2001). Susceptibility of unprotected seed and seeds of local Bambara groundnut cultivars protected with insecticidal essential oils to infestation by *Callosobruchusmaculates. Journal of Stored Products Research*, **37**, 47 - 62.

Al-Farsi, M.A. & Lee, C.Y. (2008). Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chemistry*, **108***,* 977 - 985.

Anonymous. (2010). Processing methods for dry peas, lentils & chickpeas. In: *Processing Information & Technical Manual*. Moscow: USA Dry Pea & Lentil Council.

Anonymous. (2011a). Department of Agriculture, forestry and fisheries. Directorate Production/ Plant Production. [Internet document] URL: http://www.nda.agric.za/docs/Brochures/ProdguideBambara.pdf Accessed (25/01/2014).

Anonymous. (2011b). Emulsion Stability and Testing. In: *Particle Sciences: Drug and Development Services,* **2**, 1 - 2.

AOAC. (2000). *Method 991.43 for Soluble and Insoluble Dietary Fibre*. Association of Analytical Communities: Gaithersburg, MD.

Asp, N. G. (1978). Critical evaluation of some suggested methods for assay of dietary fibre. *Journal of Plant Technology*, **3**, 21 - 25.

Azam-Ali, S., Azam-Ali, S.N., Aguilar-Manjarrez, J. & Bannayan-Avval, M. (2001a). Bambara groundnut. In: *A global mapping system or Bambara groundnut production of the Food and Agriculture Organisation of the United Nations.* Pp.1 - 3. Rome: FAO.

Azam-Ali, S. N., Sesay, A., Karikaris, S. K., Massawe, F. J., Bannayan, M. & Hampton, K. J. (2001b). Assessing the potential of an underutilised crop: A case study using Bambara groundnut. *Journal of Exploration Agriculture*, **37**, 433 - 472.

Bach Knudsen, K.E. (2001). The nutritional significance of "dietary fiber" analysis. *Animal Feed Science and Technology*, **90**, 3- 20.

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

Baryeh, E. A. (2001). Physical properties of Bambara groundnuts. *Journal of Food Engineering,* **47**, 321 - 326.

Baudoin, J. P. & Mergeai, G. (2001). Grain Legumes in crop production. In Tropical Africa. Pp. 313 - 317.

Biswas, A.K., Kumar, V., Bhosle, S., Sahoo, J. & Chatli, M.K. (2011). Dietary fibres as functional ingredients in meat products and their role in human health. *International Journal of Livestock Production*, **2**(4), 45 - 54.

Bogracheva, T., Cserhalmi, Z., Czukor, B., Fornal, J., Schuster-Gajzago, I., Kovacs, E.T., Lewandowicz, G. & Soral-Smietana, M. (2001). Processing. In: *Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomics characteristics.* (Edited by C.L. Henley). Pp. 89 - 92. New York: CABI Publishing.

Borderias, A.J., Sanchez-Alonso, I. & Perez-Mateos, M. (2005). New applications of fibres in foods: addition to fishery products. *Trends in Food Science and Technology*, **16**, 458 - 465.

Brink, M., Ramolemana, G. M. & Sibuga, K.P. (2006). *Vigna subterranean* (L.) Verdc. In: *Plant Resources of Tropical Africa: Cereals and Pulses.* (Edited by M. Brink. & G. Belay). Pp. 213 - 218. Wageningen: PROTA Foundation.

Caprita, R. & Caprita, A. (2011). Chemical Methods for the Determination of Soluble and Insoluble Non-Starch Polysaccharides – Review. *Scientific Papers: Animal Science and Biotechnologies*, **44**, 73 - 80.

Cava, R., Ladero, L., Cantero, V. & Ramirez, M.R. (2012). Assessment of Different Dietary Fibres (Tomato Fibre, Beet Root Fibre, and Inulin) for the Manufacture of Chopped Cooked Chicken Products. *Journal of Food Science*, **77**, 346 - 352.

Chanamai, R. & McClements, D.J. (2000). Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *Journal of Agricultural and Food Chemistry*, **48**, 5561.

Chanamai, R. & McClements, D.J. (2001). Depletion flocculation of beverage emulsions by gum Arabic and modified starch. *Journal of Food Science*, **66**, 457.

Chau, C.F. & Huang, Y.L. (2004). Characterization of passion fruit seed fibres - a potential fibre source. *Food Chemistry*, **85**, 189 - 194.

Cheong, K.W., Tan, C.P., Mirhosseini, H., Joanne-Kam, W.Y., Hamid, N.S.A. & Basri, M. (2014). The effect of prime emulsion components as a function of headspace concentration of soursop flavor compounds. *Chemistry Central Journal*, **8**(23), 1 - 11.

Chhabra, R.P. & Richardson, J.F. (eds) (2008). Non-Newtonian fluid behaviour. In: *Non-Newtonian flow and applied rheology: Engineering Applications.* 2nd ed. Pp. 1 - 5. Oxford: Butterworth-Heinemann Publications.

Choi, Y-S., Kim, H-W., Hwang, K-E., Song, D-H., Choi, J-H., Lee, M-A., Chunh, H-J. & Kim, C-J. (2014). Physicochemical properties and sensory characteristics of reduced-fat frankfurters with pork back fat replaced by dietary fibre extracted from *makgeolli* lees. *Meat Science*, **96**, 892 - 900.

Codex (2009). Report on the 30th session of the Codex Committee on Nutrition and Foods for Special Dietary Uses. ALINORM 09/32/26, Appendix II (p.46). Rome: Codex Alimentarius Commision.

Coultate, T.P. (2009). In: *Food: The Chemistry of its Components*; Pp 61. Cambridge: The Royal Society of Chemistry.

Cummings, J.H., Macfarlane, G.T. & Englyst, H.N. (2001). Prebiotic digestion and fermentation. *American Journal of Clinical Nutrition*, **73**, 415 - 420.

Dalgetty, D.D. & Baik, B. (2003). Isolation and characterisation of cotyledon fibres from Peas, Lentils, and Chickpeas. *American Association of Cereal Chemists*, **80**(3), 310 - 315.

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

Dhepe, P.L. & Sahu, R. (2010). A solid-acid-based process for the conversion of hemicelluloses. *Green Chemistry*, **12**, 2153 - 2156.

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.

Diedericks, C.F. (2014). Functional properties of Bambara groundnut (*Vigna Subterranea* (L) Verdc.) non-starch polysaccharides in model and food systems. Master of Technology: Food Technology Thesis, Cape Peninsula University of Technology.

Dickinson, E. (2008). Interfacial structure and stability of food emulsions as affected by protein-polysaccharide interactions. *Soft Matter,* **4**, 932 - 942.

Eckhoff, S.R., Du, L., Yang, P., Rausch, K.D., Wang, D.L. Li, B.H. & Tumbleson, M.E. (1999). Comparison between Alkali and Conventional Corn Wet-Milling: 100 g Procedures. *Cereal Chemistry*, **76**, 96 - 99.

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

Englyst, H. N., Quigley, M. E. & Hudson, G. J. (1994). Determination of dietary fiber as non-starch polysaccharides with gas-liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst*, **119**, 1497 - 1509.

European Commission. (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. *Official Journal of the European Union*, **404**, 9 - 25.

Fadaei, V. & Salehifar, M. (2012). Some chemical and functional characteristics of dietary fiber from five fiber sources. *European Journal of Experimental Biology*, **2**, 525 - 528.

FAO. (2001). Food and Agriculture Organization of the United Nations. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Pp 20.

FAO. (2011). Final 2011 *Data and preliminary 2012 Data for 5 major commodity aggregates*. Food and Agriculture Organisation of the United Nations and World Health Organisation

Fasoyiro, S.B., Ajibade, S.R., Omole, A.J., Adeniyan, O.N. & Farinde, E.O. (2006). Proximate, mineral and anti-nutritional factors of some underutilized grain legumes in South- Western Nigeria. *Nutrition and Food Science,* **38**, 18 - 23.

Fasoyiro, S., Yudi, W. & Taiwo, K. (2012). Processing and Utilization of Legumes in the Tropics. In: *Trends in Vital Food and Control Engineering*. (Edited by A.A. Eissa.). Croatia: InTech.

Fernandez-Lopez, J., Fernandez-Gines, J.M., Aleson-Carbonell, L., Sendra, E., Sayas-Barbera, E. & Perez-Alvarez, J.A. (2004). Application of functional citrus by-products to meat products. *Trends in Food Science and Technology,* **15**, 176 - 185.

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

Fischer, P. & Windhab, E.J. (2011). Rheology of food materials. *Current Opinion in Colloid and Interface Science,* **16**(1), 36 - 40.

Fotiadis, C.I., Stoidis, C.N., Spyropoulos, B.G. & Zografos, E.D. (2008). Role of probiotics, prebiotics and synbiotics in chemoprevention for colorectal cancer. *World Journal of Gastroenterology,* **14**(42), 6453 - 6457.

Furda. I. (1977). Fractionation and examination of biopolymers from dietary fibre. *Cereal Foods World*, **22**, 252 - 254.

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 Biological, Veterinary, Agricultural and Food Engineering,* **7**(11), 724-732.

Gallegos, C., Franco, J.M. & Partal, P. (2004). Rheology of food dispersions. *The British Society of Rheology,*19 - 65.

Garcia, M.L., Caceres, E. & Selgas, M.D. (2007). Utilisation of fruit fibres in conventional and reduced-fat cooked-meat sausages. *Journal of the Science of Food and Agriculture*, **87**, 624 - 631.

Garti, N. & Benichou, A. (2004). Recent developments in double emulsions for food applications. In: *Food Emulsions,* (Edited by S.E. Friberg., K. Larsson. & J, Sjoblom). New York: Marcel Decker.

Gharibzahedi, S.M.T., Mousavi, S.M., Hamedi, M. & Ghasemlou, M. (2012). Response surface modeling for optimization of formulation variables and physical stability assessment of walnut oil-in-water beverage emulsions. *Food Hydrocolloids*, **26**, 293 - 301.

Ghosh, S. & Rousseau, D. (2011). Fat crystals and water-in-oil emulsion stability. *Current Opinion in Colloid and Interface Science,* **16**(5), 421 - 431.

Gibson, G.R., Probert, H.M., Loo, J.V., Rastall, R.A. & Roberfroid, M.B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews*, **17**, 259 - 275.

Gietl, E., Mengerink, W., de Slegte, Gibson, G., Rastall, R. & van den Heuvel, E. (2012). Factors involved in the *In Vitro* fermentation of short carbohydrates in static faecal batch cultures. *International Journal of Carbohydrate Chemistry*, **2012**, 1 - 10.

Gordon, D. T. & Okuma, K. (2002). Determination of Total Dietary Fiber in Selected Foods Containing Resistant Maltodextrin by Enzymatic-Gravimetric Method and Liquid Chromatography: Collaborative Study. *Journal of AOAC International*, **85**, 435 - 444.

Graham, H., Rydberg, M. B. G. & Aman, P. (1988). Extraction of soluble dietary fibre. *Journal of Agricultural and Food Chemistry*, **36**, 494 - 497.

Gropper, S.S., Smith, J.L. & Groff, J.L. (2009). In: *Advanced Nutrition and Human Metabolism*. Pp. 166 - 121. Wadsworth, Belmont

Guillon, F. & Champ, M.M. (2000). Carbohydrate fractions of legumes: uses in human nutrition and potential for health. *British Journal of Nutrition*, **88**(3), 293 - 306.

Guillon, F. & Champ, M.M. (2002). Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International*, **33**, 233 - 245.

Hawkes C. (2006). Uneven dietary development: linking the policies of globalization with the nutrition transition, obesity, and diet-related chronic diseases. *Globalization and Health*, **2**, 4.

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

Hobden, M.R., Martin-Morales, A., Guérin-Deremaux, L., Wils, D., & Costabile, A. (2013). *In Vitro* Fermentation of NUTRIOSE® FB06, a Wheat Dextrin Soluble Fibre, in a Continuous Culture Human Colonic Model System. *PLoS ONE,* **8**(10), 77128.

Huang, Y.L., Chow, C.J. & Fang, Y.J. (2011). Preparation and physicochemical properties of fibre-rich fraction from pineapple peels as a potential ingredient. *Journal of Food and Drug Analysis*, **19**(3), 318 - 323.

Hur, S.J., Lim, B.O., Decker, E.A. & McClements, D.J. (2011). In vitro digestion models for food applicants. *Food Chemistry*, **125**, 1 - 12.

Jenkins, D.J.A., Kendall, C.W.C., Vuksan, V., Vidgen, E., Parler, T., Faulkner, D., Mehling, C.C., Garsetti, M., Testolin, G., Cunnane, S.C., Ryan, M.A. & Corey P.N. (2002). Soluble fibre intake at a dose approved by the US Food and Drug Administration for a health claim of health benefits: Serum lipid risk factors for cardiovascular disease assessed in a randomized controlled crossover trial. *American Journal of Clinical Nutrition,* **75**, 834 - 839.

Jideani, V.A. & Diedericks, C.F. (2014a). Nutritional, Therapeutic, and Prophylactic Properties of Vigna subterranea and Moringa oleifera. In: *Antioxidant-Antidiabetic Agents and Human Health*. (Edited by O. Oguntibeju). Pp 187. Croatia: InTech.

Jideani, V.A. & Diedericks, C.F. (2014b). Dietary Fibre Supplement. South Africa complete patent (2014/04371).

Kerkhofs, S., Lipkens, H., Velghe, F., Verlooy, P., Martens, J.A. (2011). Mayonnaise production in batch and continuous process exploiting magnetohydrodynamic force. *Journal of Food Engineering,* **106**(1), 35 - 39.

Khan, A.R., Alam, S., Ali, S., Bibi, S. & Khalil, I.A. (2007). Dietary fibre profile of food legumes. *Sarhad Journal of Agriculture*, **23**(3), 763 - 766.

Kone, M., Paice, A. G. & Toure, Y. (2011). Nuts and Seeds in Health and Disease Prevention. (*Edited by* V.R. Preedy*,* R. R. Watson & V. B. Patel). Pp. 189 - 196. Elsiesriver.

Kumar, R., Kumar, M.S. & Mahadevan, N. (2012). Multiple emulsions: A Review. *International Journal of Recent Advances in Pharmaceutical Research*, **2**(1), 9 - 19.

Kunzek, H., Müller, S., Vetter, S. & Godeck, R. (2002). The significance of physico chemical properties of plant cell wall materials for the development of innovative food products. *European Food Research and Technology*, **214**(5), 361 - 376.

Lawal, O.S., Adebowale, K.O. & Oderinde, R.A. (2004). Functional properties of amylopectin and amylase fractions isolated from bambarra groundnut (*Voandzeia subterranean*) starch. *African Journal of Biotechnology*, **3**(8), 399 - 404.

Lim, T.K. (2012). *Vigna subterrenea*. In: *Edible Medicinal and Non-Medicinal Plants: Volume 2, Fruits*. Pp. 961 - 962, 965. New York: Springer.

Lv, H., Hu, C., Zhong, H., Zheng, H. & Wen, C. (2012). Optimization of technology for dietary fiber extraction from Maixiansan by response surface methodology. *Chinese Medicine*, **7**, 28.

Mabhaudhi, T., Modi, A.T. & Beletse, Y.G. (2013). Growth, phenological and yield responses of a bambara groundnut (*Vigna subterranea* L. Verdc) landrace to imposed water stress: II. Rain shelter conditions. *African Journals Online*, **39**(2), 191 - 198.

Madukwe, D.K., Onuh, M.O. & Christo, I.E.C. (2011). Agronomic and Physiological Performance of Bambara Groundnut (*Vigna subterranea* (L.) Verdc) in Southeastern Nigeria. *World Journal of Agricultural Sciences*, **7**(2), 166 - 171.

Manderson, K., Pinart, M., Tuohy, K.M., Grace, W.E., Hotchkiss, A.T., Widmer,W., Yadhav, M.P., Gibson, G.R. & Rastall, R.A. (2005). In vitro determination of prebiotic properties of oligosaccharides derived from an orange juice manufacturing by-product stream. *Applied and Environmental Microbiology*, **71**(12), 8383 - 8389.

Martin, R., Jimenez, E., Heilig, H., Fernández, L., Marin, M.L., Zoetendal, E.G. & Rodríguez, J.M. (2009). Isolation of Bifidobacteria from Breast Milk and Assessment of the Bifidobacterial Population by PCR-Denaturing Gradient Gel Electrophoresis and Quantitative Real-Time PCR. *Applied Environmental Microbiolology*, **75**, 4965 - 4969.

Martin-Pelaez, S., Gibson, G.R., Martin-Orue, S.M., Klinder, A., Rastall, R.A., La Ragione, R.M., Woodward, M.J. & Costabile, A. (2008). *In vitro* fermentation of carbohydrates by porcine faecal inocula and their influence on *Salmonella* Typhimurium growth in batch culture systems. *Federation of European Microbiological Societies Microbial Ecology,* **66**, 608 - 619.

Massawe, F.J., Mwale, S.S., Azam-Ali, S.N. & Roberts, J.A. (2005). Breeding in Bambara groundnut (*Vigna subterranea* (L.) Verdc.): Strategic considerations. *African Journal of Biotechnology,* **4**(6), 463 - 471.

McClemments, D.J. & Weiss, J. (2005). Lipid emulsions. *Bailey's Industrial Oil and Fat Products,* **3***,* 14.

Mirhosseini, H., Tan, C.P., Hamid, N.S.A. & Yusof, S. (2008). Effect of Arabic gum, xanthan gum and orange oil on flavor release from diluted orange beverage emulsion, *Food Chemistry*, **107**(3), 1161 - 1172.

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

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

Mwaikambo, L.Y. (2006). Review of the history, properties and application of plant fibres. *African Journal of Science and Technology*, **7**(2), 120 - 133.

Mwale, S. S., Azam-Ali, S. N. & Massawe, F. J. (2007).Growth and development of Bambara groundnut in response to soil moisture. *Journal of Science Direct*, **26**, 345 - 353.

Murevanhema, Y.Y. (2012). Evaluation of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk fermented with lactic acid bacteria as a probiotic beverage. Masters of Technology (Food Technology) Thesis. Cape Peninsula University of Technology, South Africa.

Murevanhema, Y.Y. & Jideani, V.A. (2013). Potential of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk as a probiotic beverage: *A review. Critical Reviews in Food Science and Nutrition,* **53**(9), 954 - 967.

Ojimelukwe, P.C. (2009). Sourcing and Processing of Legumes. In: *Nigerian Agro Raw Materials Development*. (Edited by P. A. Onwualu, S. C. Obasi & U. J. Ukpabi). Abuja: Raw Materials Research and Development Council.

Ophardt, C.E. (2003). Carbohydrates (Cellulose). In: *Virtual Chembook*: *Chemisty Environmental Issues*, **103**.

O'Shea, N., Arendt, E. K. & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science and Emerging Technologies,* **16**,1 - 10.

Partal, P. & M[a] Franco, J. (2006). Non-Newtonian Fluids. *Encyclopedia of Life Support Systems.* Rheology, **1**.

Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F. & Matuchansky, C. (2005). Review article: *Bifidobacteria* as probiotic agents -- physiological effects and clinical benefits. *Alimentary Pharmacology and Therapeutics*, **22**(6), 495 - 512.

Prakongpan, T., Nitithamyong, A. & Luangpituksa, P. (2002). Extraction and Application of Dietary Fibre and Cellulose from Pineapple Cores. Journal of Food Science, **67**(4), 1308 - 1313.

Probert, H.M., Juha H. A. Apajalahti, J.H.A., Rautonen, N., Stowell, J. & Gibson, G.R. (2004). Polydextrose, Lactitol, and Fructo-Oligosaccharide Fermentation by Colonic Bacteria in a Three-Stage Continuous Culture System. *Applied Environmental Microbiology*, **70**(8), 4505 - 451.

Prosky, L., Asp, N.G., Scheweizer, T.F., DeVries, J.W. & Furda, I. (1988). Determination of insoluble and soluble, and total dietary fibre in foods and food products: Interlaboratory study. *Journal of the Association of Official Analytical Chemist*s, **71**, 1017-1023.

Qayyum, M.M.N., Butt, M.S., Anjum, F.M. & Nawaz, H. (2012). Composition analysis of some selected legumes for protein isolates recovery. *The Journal of Animal & Plant Sciences*, **22**(4), 1156 - 1162.

Ramirez, E.A., Johnston, D.B., McAloon, A.J. & Singh, V. (2009). Enzymatic corn wet milling: Engineering process and cost model. *Biotechnology for Biofuels*, **2**, 2.

S.J., Reineccius, G.A. & Peppard, T.L. (2010). A Comparison of the Stability of Beverage Cloud Emulsions Formulated with Different Gum Acacia- and Starch-Based Emulsifiers. *Journal of Food Science*, **75**, 236 - 246.

Roberfroid, M. (2007). Prebiotics: The concept revisited. *Journal of Nutrition*, **137**, 830 - 837.

Robins, M.M., Watson, A.D. & Wilde, P.J. (2002). Emulsions – creaming and rheology. *Current Opinion in Colloid and Interface Science,* **7**, 419 - 425.

Rodriguez, R. A. J., Fernandez-Bolanos J., Guillen R. & Heredia A. (2006). Dietary Fibre from Vegetable Products as a Source of Functional Ingredients. *Trends in Food Sciences and Technology*, **17**, 3 - 15.

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.

Rycroft, C.E., Jones, M.R.M., Gibson, G.R. & Rastall, R.A. (2001). A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *Journal of Applied Microbiology,* **91**, 878 - 887.

Sadiku-Agboola, O., Sadiku, E.R., Adegbola, A.T. & Biotidara, O.F. (2011). Rheological Properties of Polymers: Structure and Morphology of Molten Polymer Blends. *Materials Sciences and Applications*, **2**, 30 - 41.

Sahin, S. & Sumnu, S.G. (2006). Rheological properties of foods. In: *Physical properties of foods*. Pp. 39 - 105. Turkey: Springer.

Salehifar M. & Fadaei V. (2011). Comparison of some functional properties and chemical constituents of dietary fibers of Iranian rice bran extracted by chemical and enzymatic methods. *African Journal of Biotechnology*, **8**, 18525 - 18531.

Sannasiddappa, T.H., Costabile, A., Gibson, G.R. & Clarke, S.R. (2011). The Influence of *Staphylococcus aureus* on Gut Microbial Ecology in an *In Vitro* Continuous Culture Human Colonic Model System. *PLoS ONE,* **6**(8), 23227.

Shanmugasundaram, S. (2003). Report of the APO Seminar on Processing and Utilization of Legumes, Japan. Asian Productivity Organization.

Shegro, A., Van Rensburg, W. S. & Adebola, P. O. (2013). Assessment of genetic variability in bambara groundnut (*Vigna subterrenea* (L). Verdc.) using Morphological Quantitative traits. *Academic Journal of Agricultural* Research, **1**(3), 45 - 51.

Sivam, A.S., Sun-Waterhouse, D., Quek, SY. & Perera, C.O. (2010). Properties of Bread Dough with Added Fibre Polysaccharides and Phenolic Antioxidants: A Review. *Journal of Food Science*, **75**(8), 163 - 174.

Slavin, J. (2013). Fibre and Prebiotics: Mechanisms and Health Benefits. *Nutrients*, **5**(4), 1417 - 1435.

Southgate, D. A. T. (1969). Determination of carbohydrates in foods: Unavailable carbohydrates. *Journal of the Science of Food and Agriculture,* **20**, 331 - 335.

Stern, P., Velentova, H. & Pokorny, J. (2001). Rheological properties and sensory texture of mayonnaise. *European Journal of Lipid Science and Technology,* **103**(1), 23 - 28.

Tadros, T. (2013). Creaming of emulsions. In: *Encyclopedia of Colloid and Interface Science.* (Edited by T. Tadros). Berlin Heidelberg: Springer.

Tomaschunas, M., Zorb, R., Fischer, J., Kohn, E., Hinrichs, J. & Busch-Stockfisch, M. (2013). Changes in sensory properties and consumer acceptance of reduced fat pork Lyon-style and liver sausages containing inulin and citrus fibre as fat replacers. *Meat Science*, **95**, 629 - 640.

Trumbo, P., Schlicker, S., Yates, A.A. & Poos, M. (2003). Dietary reference intakes for energy, carbohydrate, fibre, fat, fatty acids, cholesterol, protein and amino acids. *Journal of American Dietetic Association*, **103**(5), 563.

Vanholme, R., Demedts, B., Morreel, K., Ralph, J. & Boerjan, W. (2010). Lignin biosynthesis and structure. *Plant physiology*, **153**, 895 - 905.

Verma, A.K. & Banerjee, R. (2010). Dietary fibre as functional ingredient in meat products: a novel approach for healthy living – A review. *Journal of Food Science and Technology*, **47**(3), 247 - 257.

Vigsnæs, L.K., Holck, J., Meyer, A.S. & Licht, T.R. (2011). *In Vitro* fermentation of sugar beet Arabino-Oligosaccharides by fecal microbiota obtained from patients with ulcerative colitis to selectively stimulate the growth of *Bifidobacterium* spp and *Lactobacillus* spp. Applied and Environmental Microbiology, **77**(23), 8336 - 8344.

Wan, Y., Espinoza Rodezno, L.A., Mis Solval, K., Juan Li, J. & Sathivel, S. (2002). Optimization of Soluble Dietary Fiber Extraction from Defatted Rice Bran using Response Surface Methodology. *Journal of Food Processing and Preservation,* **38**, 441 - 448.

Westenbrink, S., Brunt, K. & Van de Kamp, J.W. (2012). Dietary fibre: Challenges in production and use of food composition data. *Food Chemistry*, **140**, 562 - 567.

Wood, J.A. & Grusak, M.A. (2007). Nutritional value of chickpea. In: *Chickpea breeding and management*. (Edited by S.S. Yadav, R. Redden, W. Chen & B. Sharma). Pp.119. Trowbridge: Cromwell Press.

Yadav, M.P., Johnston, D.B., Hotchkiss, A.T. & Hicks, K.B. (2007). Corn fibre gum: A potential gum arabic replacer for beverage flavor emulsification. *Food Hydrocolloids*, **21**(7), 1022 - 1030.

Yang, S.C. & Lai, L.S. (2003). Dressing and mayonnaise. *Encyclopedia of Emulsion Technology.* (Edited by B. Cabellero., L.C. Trugo., P.M. Finglas). London: Academic Press.

Yangilar, F. (2013). The Application of Dietary Fibre in Food Industry: Structural Features, Effects on Health and Definition, Obtaining and Analysis of Dietary Fibre: A Review. *Journal of Food and Nutrition Research,* **3**, 13 - 23.

Zhang, J. (2011). Novel emulsion-based delivery systems. PhD Thesis, University of Minnesota.

CHAPTER THREE

PHYSICOCHEMICAL AND RHEOLOGICAL CHARACTERISTICS OF BAMBARA GROUNDNUT DIETARY FIBRES EXTRACTED USING THE WET MILLING METHOD

Abstract

Insoluble dietary fibres (IDF) and soluble dietary fibres (SDF) from four varieties of BGN seeds were extracted through dry milling and wet fractionation. The physicochemical, thermal and rheological properties of BGN SDFs and IDFs were assessed. The yield of SDFs ranged from 15.4% (black-eye and red) to 17.1% (brown-eye and brown) and the IDF yield ranged from 12.0% (brown-eye) to 15.6% (red). There was no significant ($p > 0.05$) difference in the yield of the SDFs as well as among the IDFs of BGN. The swelling capacities of brown-eye (6.5 g/mL) and black-eye IDFs (6.2 g/mL) were significantly ($p < 0.05$) higher than those of red (6.0 g/mL) and brown IDFs (5.5 g/mL). The water holding capacities (WHCs) of black-eye and brown-eye IDFs (2.84 mL water/g sample and 2.83 mL water/g sample, respectively) were significantly ($p < 0.05$) higher than the WHCs of brown and red IDFs. The bulk densities of BGN fibres ranged from 0.57 g/mL (red IDF) to 0.67 g/mL (brown-eye IDF) for IDFs and from 0.46 g/mL (brown-eye) to 0.57 g/mL (black-eye) for SDFs. The oil binding capacities (OBCs) of SDFs ranged from 2.78 g oil/g sample (brown) to 4.03 g oil/g sample (brown-eye). The OBC of brown-eye SDF was significantly ($p < 0.05$) higher than those of the other three SDFs. The OBC of all four IDFs did not differ significantly ($p > 0.05$), ranging from 1.40 g oil/g sample (brown-eye and black-eye) to 1.52 g oil/g sample (brown). Brown-eye and black-eye IDFs were lighter (L*), redder (+a*), yellower (+b*), more saturated and had a higher hue compared to the red and brown IDFs. All four SDFs differed significantly ($p < 0.05$) from each other in their hydrolysable polyphenolic (HPP) content ranging from 6.89 mg/g gallic acid equivalent (GAE) for brown SDF to 20.86 mg/g GAE for brown-eye SDF. IDFs had a HPP content ranging from 10.96 mg/g GAE (black-eye) to 14.43 mg/g GAE (brown). The least and highest tannin contents were reported for brown IDF (1.1 mg/g) and black-eye (2.1 mg/g), respectively. The total sugar composition of the BGN fibres ranged from 31.2% (red) to 44.8% (black-eye) in SDFs and from 23.8% (black-eye) to 25.8% (brown) in IDFs. All SDFs exhibited shear thinning behaviour and exhibited exothermic enthalpies. Wet milling extraction proved to be a cost effective alternative to the enzymatic-gravimetric method.

3.1 Introduction

Bambara groundnut (BGN) is an underutilised crop predominantly grown in African countries (Jideani & Mpokotwane, 2009; Jideani & Diedericks, 2014). Legume seeds such as BGN are good sources of metabolically active soluble dietary fibre (SDF) and total dietary fibre as a whole (Fasoyiro *et al*., 2006). BGN dietary fibre has potential for use as a food ingredient, fibre supplement, fortifying agent and also in non-food applications such as animal feed and pharmaceuticals (Fasoyiro *et al*., 2012). An increase in consumer awareness of the health benefits of dietary fibre (DF) has led to the investigation of alternative sources and applications of DF by a number of researchers (Dalgetty & Baik, 2003; Ojimelukwe, 2009; Sivam *et al.*, 2010; Daou & Zhang, 2011; Dhingra *et al.*, 2012; Yangilar, 2013; Bojnanska *et al.*, 2014; Diedericks, 2014). Dietary fibre reduces constipation and some lifestyle diseases such as obesity, diabetes, coronary heart diseases, some cancers and piles (Hawkes, 2006; Wood & Grusak, 2007; Daou & Zhang, 2011; Rakha, 2011).

Legumes that have been researched for dietary fibre extraction include cowpeas, lentils, chickpeas, pigeon peas, green peas and kidney beans (Dalgetty & Baik, 2003; Khan *et al*., 2007). Several methods of extracting DF from various sources are available. The basis of all the methods is similar however the approach differs depending on the end use of the DF, source of dietary fibre and availability of equipment. All the methods of extracting fibre involve fractionation; this allows for the isolation and seperation of components to obtain the desired concentrates (Anon., 2010). Some methods of extracting DF include microbiological retting, chemical methods, enzymatic methods, dry processing and wet processing methods (Bogracheva *et al*., 2001).

Wet processing methods involve the use of water to produce products with a high purity that can be used for a wide range of applications (Bogracheva *et al*., 2001). Using these methods, protein is extracted at an alkaline pH from the finely ground flour (Bogracheva *et al*., 2001; Dalgetty & Baik, 2003; Anon., 2010). The protein extract is separated from the insoluble DF fraction using acid precipitation or ultra-filtration (Anon., 2010). The separation of insoluble extract is based on differences in swelling properties of dietary fibre and starch (Bogracheva *et al*., 2001). At room temperature DF has a relatively high swelling capacity whilst the swelling of starch is very restricted. The insoluble extract fraction is dispersed in a large amount of water and screened through a series of sieves with pore diameters ranging from 30 - 300 µm (Bogracheva *et al*., 2001; Dalgetty & Baik, 2003). The supernatant is mainly a dispersion of starch granules and the residue is mainly DF (Dalgetty & Baik, 2003; Anon., 2010). The extracted DFs are then spread to dry at temperatures above 100°C or preferably freeze-dried (Bogracheva *et al*., 2001; Anon., 2010).

The modification of the wet milling method as reported by Dalgetty & Baik (2003) is more efficient than the conventional wet methods that rely solely on the differences in swelling capacity to separate starch and DFs, as it makes use of the enzyme α-amylase to

digest any remaining starch thus purifying the DF concentrate. This modified wet milling method was adopted in this work. Extracted DF from BGN using the wet milling method is not documented. Furthermore the properties and application of BGN DF have also not been widely documented. An understanding of the physicochemical, thermal, structural and rheological properties of BGN DF will highlight its behaviour in different food and non-food systems including in the human gastro-intestinal tract (Tiwari & Cummins, 2011; Urriola *et al.*, 2013).

Diedericks (2014) applied an enzymatic-gravimetric method of extracting DF from BGN. The method proved to be very costly and time consuming costing approximately ZAR26388.57/kg DF for 56 extractions. The objectives of this chapter were to (1) extract soluble and insoluble dietary fibres from whole seeds of BGN using the wet milling method as an alternative to the enzymatic-gravimetric method; (2) evaluate their physicochemical, rheological, structural and thermal properties and (3) compare the cost of the wet milling method used in this study to the enzymatic-gravimetric method reported by Diedericks (2014).

3.2 Materials and Methods

3.2.1 Source of materials

BGN seeds were purchased from Triotrade in Johannesburg, South Africa and sorted into four varieties according to 'eye' colour namely, the black-eye, brown-eye, brown and red. Figure 3.1 outlines the different analyses that were carried out in this chapter. Chemicals used in this study were of analytical grade (Sigma-Aldrich, Johannesburg, South Africa). Equipment used in this study was obtained from the Departments of Food Technology, Oxidative Stress and Chemical Engineering of the Cape Peninsula University of Technology and the Scanning Electron Microscope Unit of Stellenbosch University, South Africa.

3.2.2 Milling of BGN seeds

Whole seeds (hulls and cotyledons) seeds were washed and dried at 50°C for 48 h (Cabinet drier, Model: 1069616). The seeds were then milled using a hammer mill (Trapp TRF 40, Animal ration shredder/Hammer mill foliage, Jaraqua do sul-sc, Brasil) with a sieve size of 250 µm. The flour was stored in clear plastic bags at refrigeration temperature (4 - 6°C) until used.

3.2.3 Wet fractionation of BGN flour into individual constituents

The method of Dalgetty & Baik (2003) was adopted in this study. BGN flour (200 g) was mixed with 500 mL distilled water and blended for 3 min at the highest setting (Figure 3.2).

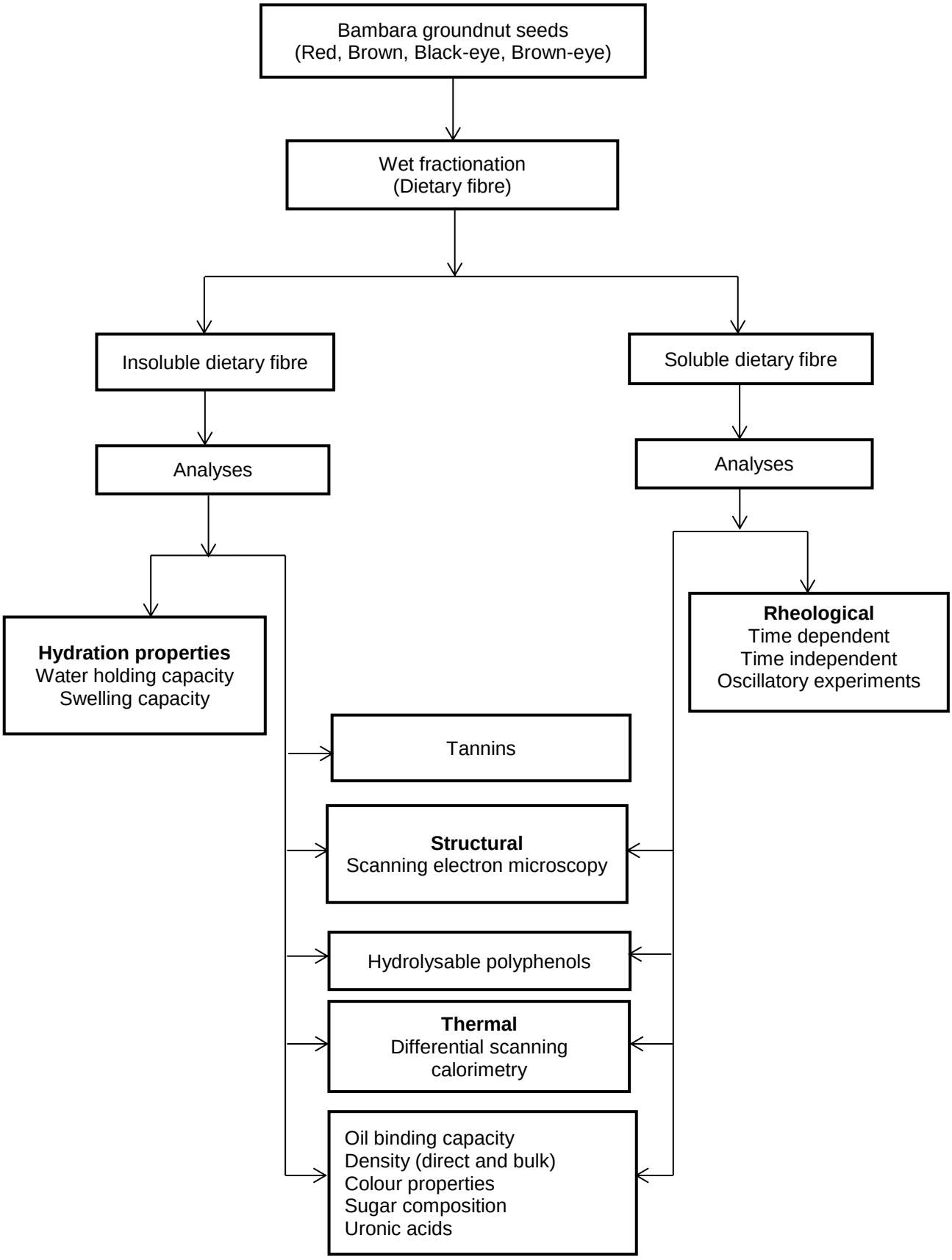

Figure 3.1 Experimental design for Chapter 3.

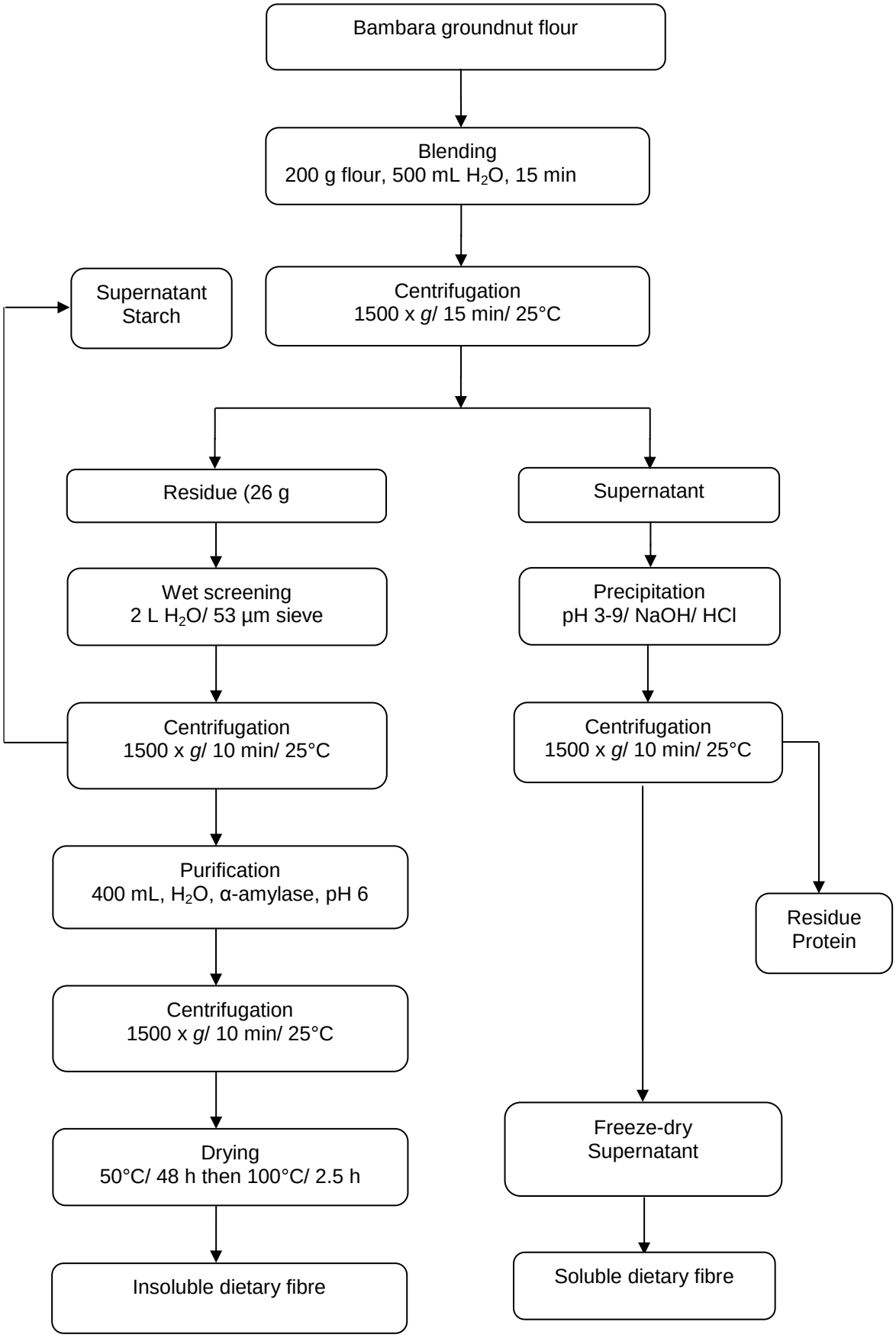

Figure 3.2 Process flow for isolation of Bambara groundnut dietary fibres.

The slurry was subjected to centrifugation (Avanti® J-E Centrifuge, JSE111330, Beckman Coulter Inc., USA) for 15 min at 25°C at a speed of 1500 x *g* twice. The residue was used for the isolation of IDF and the supernatant was used in the isolation of SDF. The details of the extraction methods for soluble and insoluble BGN DF are described in the following sections.

1. *Isolation of insoluble dietary fibre*

The residue (26 g) was wet screened in 2 L of water through a 53 µm sieve. The supernatant was collected as starch concentrate. To purify IDF, the collected pellet was placed into plastic fibre incubation flasks (600 mL) and digested using 13 units/mg 10 MU heat-stable α-amylase in 400 mL of water at pH 6 for 30 min, in a shaking water bath (100°C). After digestion; the tubes were left to cool down to room temperature and centrifuged (10 min, 25°C, 1500 x *g*). The residue was collected and dried at 50°C (Cabinet drier, Model: 1069616) for 48 h then vacuum dried in an air oven at 100°C for 2.5 h.

2. *Isolation of soluble dietary fibre*

The first step in the isolation of SDF was the precipitation of protein by adjusting the pH of the soluble fraction from pH 3 to pH 9 using 1 *N* NaOH and 1 *N* HCl. Following precipitation, the soluble fraction was centrifuged (10 min, 25°C, 1500 x *g*). The supernatant was filtered against Millipore water according to the method of Diedericks (2014). The supernatant was subjected to a tangential flow filtration system (Spectrum Laboratories Inc., USA) and each fibre solution was washed with four diafiltration volumes of Millipore water to remove any contaminants. Waste was removed through a hollow fibre filtration outlet with a molecular weight cut off of 10 kD (Diedericks, 2014). The recovered SDF was freeze-dried by placing 600 mL of each sample in trays that were loaded onto the freeze dryer shelf. The freeze-dryer shelf temperature was dropped to 5°C, then decreased to -5°C after 30 min and held at that temperature for 30 min, then further dropped to -10°C for another 30 min to ensure uniform super-cooling. The temperature was then decreased to -57°C to initiate the freezing step. The freeze drying process was completed in 24 h.

3.3 Physicochemical Properties of BGN Dietary Fibres

BGN insoluble and soluble dietary fibres were evaluated for oil binding capacity (OBC), density (bulk and direct), rheological and thermal properties, colour, sugar composition, structure, particle size distribution and polyphenolic compounds. BGN IDFs were further evaluated for swelling capacity, water holding capacity (WHC) and tannins.

3.3.1 Hydration properties of BGN dietary fibres

1. *Swelling capacity of BGN dietary fibres*

The swelling capacities (SWC) of BGN IDFs were determined using the method of Wang & Toews (2011). Dry, purified IDF (0.2 g) was hydrated with 10 mL of distilled water in a graduated cylinder and left to stand at room temperature for 18 h. Swelling capacity was then calculated as the volume occupied by the sample divided by the original weight of the sample (0.2 g) and expressed as mL/g as shown by equation 3.1.

$$\text{Swelling capacity } \left(\frac{\text{mL}}{\text{g}}\right) = \frac{\text{Volume occupied by sample (mL)}}{\text{Original sample weight (g)}} \qquad \textit{Equation 3.1}$$

2. *Water holding capacity of BGN dietary fibres*

The water holding capacity (WHC) of BGN IDFs was determined using the method described by Dalgetty & Baik (2003) with modifications. In a 50 mL centrifuge tube, 1 g of IDF and 30 mL of deionised water were added and the tubes were held for 18 h at 24°C to allow sufficient hydration of the fibre. The tubes were then centrifuged (3000 x *g*, 20 min, 23°C) (Centrifuge, Model MR 812, Jonan, Thermo electron corporation). The supernatant was decanted and the tubes carefully inverted for 10 min to drain any remaining free water. Water holding capacity was then calculated as the amount of water retained by the fibres (difference between original volume of water and the volume of the supernatant) divided by the original weight of the dry sample. The WHC was calculated using equation 3.2 and expressed as mL of water/g dry sample.

$$\text{Water holding capacity } \left(\frac{\text{mL}}{\text{g}}\right) = \frac{\text{Water retained (mL)}}{\text{Original dry sample weight (g)}} \qquad \textit{Equation 3.2}$$

3.3.2 Density of BGN dietary fibres

The method of Parrott & Thrall (1978) was used to determine both bulk and direct densities. The procedures followed in the analyses of bulk and direct densities are discussed in the following sections.

1. *Bulk density*

Bulk density was determined by adding 2 g of each BGN fibre into a graduated syringe and manually applying sufficient pressure while gentle tapping the syringe on a bench until the contents were packed tightly. This was done until the volume could not be reduced any further by any additional pressure. Bulk density was then calculated as the original weight of the fibre divided by the final volume of the fibre in the syringe and was expressed as g/mL (Equation 3.3).

$$\text{Bulk density } \left(\frac{g}{mL}\right) = \frac{\text{Original weight of fibre (g)}}{\text{Final volume of fibre (mL)}} \qquad \textit{Equation 3.3}$$

2. *Direct density*

Fibre was added to the 5 mL mark in a 10 mL graduated cylinder. Care was taken to avoid shaking the cylinder so as to avoid settling of the fibre. The fibre was then emptied and weighed. Direct density was calculated as the weight of fibre divided by the final volume of fibre in the syringe and was expressed as g/mL (Equation 3.4).

$$\text{Direct density } \left(\frac{g}{mL}\right) = \frac{\text{Weight of fibre (g)}}{\text{Final volume of fibre (mL)}} \qquad \textit{Equation 3.4}$$

3.3.3 Oil binding capacity of BGN dietary fibres

The method described by Dalgetty & Baik (2003) was applied in the determination of the oil binding capacities (OBC) of BGN fibres with modifications. Fibre (1 g) was mixed with 5 g of canola oil in a 50 mL centrifuge tube. The mixture was vortexed for 30 sec at 5 min intervals for 30 min. The mixture was then centrifuged (Centrifuge, Model MR 812, Jonan, Thermo electron corporation) at 1600 x *g* for 25 min at 23°C. After centrifugation the supernatant (free oil) was decanted and weighed. The difference between the original weight of oil (5 g) and the weight of the decanted oil was considered as retained oil. Oil binding capacity was calculated using equation 3.5 as weight of oil retained divided by the original weight of the fibre.

$$\text{Oil binding capacity } \left(\frac{g}{g}\right) = \frac{\text{Weight of oil retained (g)}}{\text{Weight of fibre (g)}} \qquad \textit{Equation 3.5}$$

3.3.4 Colour measurements of BGN dietary fibres

Colour characteristics of BGN dietary fibres were determined using spectrophotometry (Model CM-5, Konica Minolta Sensing, Japan) set at standard observer 10° and D65. The spectrophotometer was calibrated with a black tile (L* = 5.49, a* = 7.08, b* = 4.66) and a white tile (L* = 93.41, a* = 1.18, b* = 0.75) followed by zero calibration. BGN fibre (3 g of IDF and 0.6 - 0.8 g SDF) were individually placed in a glass sample holder (diameter 30 mm). L*, a* and b*, hue and chroma were assessed using L*C*h* and CIE-L*a*b* colour space systems. Each variety was analysed in triplicates. Colour differences (ΔE) amongst the fibre samples were calculated using the colour difference equation (Khen, 2002) (Equation 3.6).

$$\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})} \qquad \textit{Equation 3.6}$$

Where: L*: lightness; a*: redness/greenness; b*: yellowness/blueness

3.3.5 Microstructural analysis of BGN dietary fibres

The microstructure and particle size distribution of BGN fibres was analysed using a scanning electron microscope (SEM, Leo$^{(R)}$ 1430VP). Prior to analysis, the samples were carefully mounted on aluminium stubs with double sided carbon tape. Each sample was then coated with a thin layer of gold to make it electrically conducting. Beam conditions during imaging were 7 kV. Two fields per sample were studied to obtain a representative number of particles (between 300 and 800 particles) (Prakongpan *et al*., 2002; Rosell *et al*., 2009). Image J v1.36b software was used to analyse the particle sizes from the micrograms.

Particle sizes and particle size distributions of each BGN fibre was assessed using images obtained from the SEM. The diameters of the particle sizes (n = 50) were measured individually according to the method of Tcholakova *et al.* (2004). Fibre particle sizes were obtained in terms of volume-surface mean diameter ($d_{3,2}$) (Equation 3.7) and equivalent volume-mean diameter ($d_{4,3}$) (Equation 3.8).

$$d_{3,2} = \frac{\sum n_i d_{i3}}{\sum n_i d_{i2}} \qquad \textit{Equation 3.7}$$

$$d_{4,3} = \frac{\sum n_i d_{i4}}{\sum n_i d_{i3}} \qquad \textit{Equation 3.8}$$

Where n_i is the number of particles with diameter d_i.

3.3.6 Assessment of polyphenolic compounds in BGN dietary fibres

The method of Diedericks (2014) was adopted in the assessment of polyphenolic compounds. Proanthocyanidins (condensed tannins) and hydrolysable polyphenols (HPPs) were determined in BGN fibres. Condensed tannins were determined only in IDFs while hydrolysable polyphenols were determined in both SDFs and IDFs. The details of the assessments of these phenolic compounds are discussed in the following sections.

1. *Determination of hydrolysable polyphenols in BGN dietary fibres*

BGN IDF samples (250 mg) were mixed with 10 mL of methanol and 1 mL of H_2SO_4 in 14 mL centrifuge tubes. The samples were then incubated at 80°C for 20 h. After incubation, the samples were centrifuged (4000 x *g,* 5 min, 21°C) and the residues were collected for HPP analysis using the Folin-Ciocalteu assay by mixing 25 µL of sample with 125 µL of 0.2 M Folin-Ciocalteu and 100 µL of 7.5% Na_2CO_3 solution. The mixtures were left to stand for 2 h

then absorbance was measured using a spectrophotometer at a wavelength of 750 nm using a gallic acid standard calibration curve. The results were expressed as mg/g gallic acid equivalents (GAE). For determination of HPPs in BGN SDFs, samples (250 mg) were dissolved in 10 mL distilled water, centrifuged (4000 x *g,* 5 min, 21°C) and the supernatant was subjected to Folin-Ciocalteu assay as described above.

2. *Determination of condensed tannins in BGN dietary fibres*

Samples (250 mg) were treated with 5 mL/L HCl-Butanol mixed in a 1:1 ratio. The mixture was incubated at 100°C for 1 h then centrifuged (4000 x *g,* 5 min, 21°C). Condensed tannins were calculated from the anthocyanidin solutions absorbance at a wavelength of 555 nm using a standard curve of 0.0072 ppm and an absorbance of +0.0072.

3.3.7 Assessment of neutral sugars and uronic acids in BGN dietary fibres

BGN fibres were subjected to acid hydrolysis prior to analysis for sugars and acids. BGN SDF samples (50 mg) were hydrolysed with 1 M H_2SO_4 at 100°C for 90 min and IDFs were first hydrolysed with 12 M H_2SO_4 at 30°C for 90 min then with 1 M H_2SO_4 at 100°C for 90 min to yield monomers. After hydrolysis, samples were centrifuged (3000 x *g,* 15 min, 21°C). IDF residues were washed twice with 2 mL of distilled water while SDF supernatants were filtered to remove any suspensions. Uronic acids and neutral sugars were then analysed in the IDF and SDF supernatants by spectrophotometry (340 nm) using K-Arga, K-Fucose, K-Mangl, K-Rhan, K-Uronic and K-Xylose assay kits (Megazyme International, Ireland).

3.3.8 Assessment of rheological properties of BGN dietary fibres

The rheological properties of BGN SDFs were evaluated using an MCR 300 Paar Physical rheometer (Discovery HR-1, hybrid rheometer). Four varieties of BGN SDFs (black-eye, brown-eye, brown, red) were prepared into 4, 6, 8, 10, 12 and 14% concentrations by blending the samples with deionised water for 1 min in a Waring blender at the highest setting. The slurries were left to equilibrate at room temperature for 5 min. The shear-dependent/time independent, time dependent and viscoelastic properties of BGN SDFs were then evaluated. All measurements were conducted at 20°C.

1. *Time dependent rheological analyses*

Time dependent rheological experiments of the SDFs were conducted without previous shearing. Samples (25 mL) were carefully transferred into the rheometer cup and allowed to equilibrate for 5 min. The change in viscosity was then measured as a function of increasing shear rate from 0.01 to 1000 s^{-1} followed by a decreasing rate from 1000 to 0.01 s^{-1}. In order to describe the time dependent flow behaviour, experimental data (shear stress-shear rate) of forward and backward curves were fitted to Power law model. The hysteresis loop area

(Equation 3.9) was calculated as the area between the upstream data and downstream data (Tarrega *et al.,* 2004; Koocheki & Razavi, 2009).

$$\int_{\gamma_1}^{\gamma_2} K\gamma^{n} - \int_{\gamma_1}^{\gamma_2} K'\gamma^{n'} \qquad \textit{Equation 3.9}$$

Where, K, K' are the consistency coefficients and n, n' are the flow behavior indices for upward and downward measurements, respectively. Each experiment was performed in duplicate.

2. *Time independent rheological analyses*

The response of the viscosity of BGN SDFs suspensions to variations in shear rate was evaluated (Sahin & Sumnu, 2006). To conduct steady state shear experiments, samples (25 mL) were carefully transferred into the rheometer cup and allowed to equilibrate for 5 min before shearing. Fibre viscosity was measured over a shear rate range of 0.01 – 500 s^{-1} for a duration of 300 s. All measurements were performed in duplicates. Experimental flow data were evaluated and fitted to Power law as expressed by Izidoro *et al.* (2009) (Equation 3.10).

$$\tau = K\gamma^{n} \qquad \textit{Equation 3.10}$$

Where: n = flow behaviour index which indicates the tendency of a fluid to shear thinning, *K* = consistency efficiency, γ = shear rate, τ = shear stress.

3. *Oscillatory analyses*

Oscillatory experiments were conducted on all concentrations (4, 6, 8, 10, 12 and 14%) of the four BGN SDFs. Fibre solutions were carefully transferred into the rheometer cup and allowed to rest for 5 min before rheological assessment proceeded. Frequency sweep experiments were conducted at constant frequency of 1 Hz and the storage and loss moduli were recorded as a function of stress (0.01 – 100 Pa). In order to determine the linear viscoelastic region, the storage modulus was plotted against shear stress. A shear stress of 0.1 Pa was afterwards selected for further frequency sweep experiment. Frequency sweep experiment was conducted over a frequency range of 0.63 – 62.8 rad/s at a constant stress of 0.1 Pa. The fingerprint of each BGN SDF sample in terms of storage and loss moduli was then plotted as a function of frequency. All experiments were conducted in duplicate.

3.3.9 Assessment of the heat stability of BGN dietary fibres using DSC

The changes in the physical properties of BGN fibres as a function of temperature and time were assessed using Differential Scanning Calorimetry (DSC) (Perkin Elmer, DSC 6000, United Kingdom) according to a modified method of Gill *et al*. (2010). The adsorption of heat by the fibres at a temperature range of 30°C - 450°C at a rate of 5°C was assessed. A sample (2 mg) was placed in a DSC pan. The pan was positioned directly above a constantan disc and the sample temperature was measured by a chromel-alumel thermocouple. An empty pan was used as a reference. The temperature difference across the sample and the reference chromel wafers gave a measurement of heat flow. Pure Indium standard was used for calibration and the experiments were conducted in duplicate. The thermal behaviour of BGN fibres was observed as peaks. The onset, peak and end of each peak was captured as well as the enthalpy change of reaction.

3.3.10 Data analysis

IBM Statistical Package for the Social Science (IBM SPSS, version 22, 2013) was used for data analysis. Values were expressed as mean ± standard deviation. The results were subjected to Multivariate Analysis of Variance (MANOVA) to determine mean differences between treatments. Duncan's multiple range test was conducted to separate means where differences existed. To describe rheological data so as to predict the behaviour of BGN fibres, Power law rheological model was used (Equation 3.10).

3.4 Results and Discussion

3.4.1 Yield of BGN dietary fibres

The objective of isolating soluble and insoluble BGN dietary fibres using the modified wet milling method of Dalgetty & Baik (2003) was successfully attained and the yield of each fibre is given in Table 3.1. Figure 3.3 shows BGN SDFs and IDFs. The yield of SDFs was between 15.4% (black-eye and red) and 17.1% (brown-eye and brown). There was no significant ($p > 0.05$) difference in the yield of all four BGN varieties. According to the Foodstuffs, Cosmetics and Disinfectant Act, 1972 (Revised 1 March 2010) a food substance is high in fibre if it contains more than 6 g per 100 g dry solids. Hence, all four BGN varieties were good sources of SDF and were considered 'high in soluble dietary fibre'. The SDF content of most edible legumes such as pea, broad pea and soybean cotyledons range between 3.3% and 13.8% (Guillon & Champ, 2002; Khan *et al*., 2007).

The yield of SDFs in this study was higher than that of pea, broad pea and soybean which is an indication that BGN is superior in SDF content than these legumes and therefore could have a market advantage over them.

Table 3.1 Yield of dietary fibres from four Bambara groundnut varieties

Variety	SDF (%)	IDF (%)	TDF (%)
Black-eye	15.4 ± 1.71[a]	13.1 ± 2.46[a]	28.6[a]
Brown-eye	17.1 ± 3.30[a]	12.0 ± 1.40[a]	29.0[a]
Red	15.4 ± 0.93[a]	15.6 ± 0.93[a]	31.0[a]
Brown	17.1 ± 0.62[a]	13.7 ± 2.67[a]	30.7[a]

Values are mean ± standard deviation. Means within a column followed by the same subscript are not significantly [$p > 0.05$] different. SDF: Soluble dietary fibre; IDF: Insoluble dietary fibre; TDF: Total dietary fibre.

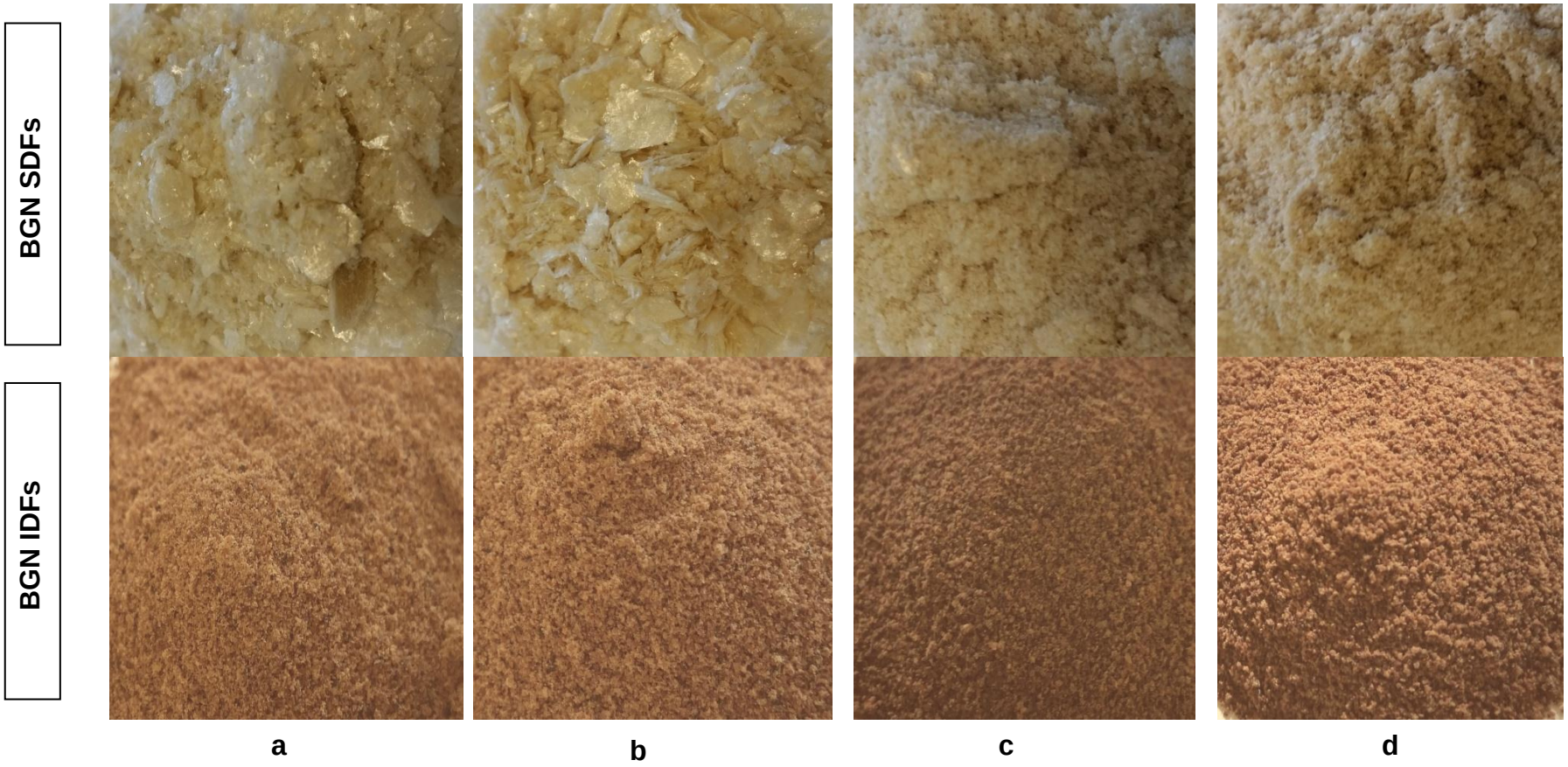

Figure 3.3 Soluble and insoluble dietary fibres (SDFs and IDFs, respectively) isolated from four varieties of Bambara groundnut (BGN) (a) Black-eye (b) Brown-eye (c) Brown (d) Red.

The higher yield of BGN fibre compared to pea and lentils may be attributed to the sugar composition of the different legumes. Arabinose was reported absent in pea and lentil SDFs (Dalgetty & Baik, 2003) while 9.4 - 19.6% were reported in BGN SDFs. Galactose and xylose were also reported absent in pea and lentil IDFs while high amounts were reported for BGN IDFs with galactose in the range 2.3 - 2.8% and xylose in the range 11.3 - 13.8%. In addition, low uronic acid contents were reported in pea and lentil SDFs (1.0 - 1.3%) and IDFs (1.7 - 2.8%) while higher uronic acid contents were reported in BGN SDFs (10.6 - 11.5%) and IDFs (6.7 - 10.6%). The sugar composition of fibres affects their yield, structure and behaviour.

Using the enzymatic-gravimetric method, Diedericks (2014) reported the yield of four varieties of BGN SDFs in the range 1.3% (brown) to 3.5% (black-eye). The lower yield of BGN DFs obtained using the enzymatic-gravimetric method may be attributed to several factors. The enzymatic-gravimetric method subjects a sample to a succession of enzymes (α-amylase, pancreatin, pepsin and amyloglucosidase), buffers (sodium acetate, phosphate buffer and trizma-maleate) and chemicals (sodium hydroxide, hydrochloric acid and ethanol). Dietary fibre is lost in each step throughout processing. The repeated processing of a sample through the steps of the enzymatic-gravimetric method results in a low recovery of dietary fibre. Furthermore, chemicals used in this method solubilise some of the components of dietary fibre such as lignin and most SDFs resulting in a low yield of dietary fibre (Gordon & Okuma, 2002).

The yield of IDF was in the range 12.0% (brown-eye) to 15.6% (red). There was no significant difference ($p > 0.05$) among all four IDFs. All four BGN varieties were good sources of IDF (Foodstuffs, Cosmetics and Disinfectant Act, 1972). Dalgetty & Baik (2003) applied the wet milling method for fibre extraction in peas, lentils and chickpeas and reported lower values (10.0% - 11.4%) of fibre to those of this study. This was an indication that BGN is superior in fibre content than peas, lentils and chickpeas. Diedericks (2014) reported relatively lower BGN IDF values in the range 6.6% (brown-eye) to 11.7% (red). Differences in IDF yield could be due to different extraction methods, the enzymatic-gravimetric method results in the loss of some IDFs which could explain the lower yield (Gordon & Okuma, 2002).

Total dietary fibre (TDF) was highest in the red variety (31.0%) and lowest in the black-eye variety (28.6%) as shown in Table 3.1. Diedericks (2014) reported lower TDF of BGN seeds in the range 17.7% (brown-eye) to 23.9% (black-eye). The yield of BGN in this present study was considered high as several researchers have reported BGN DF content in the range 5.2% and 6.4% (Baryeh, 2001; Mkandawire, 2007; Murevanhema & Jideani, 2013). The variations in yield among researchers may be attributed to differences in BGN varieties, species, climatic conditions, type of soil grown on, processing and determination methods. Higher TDF values in this study could also be attributed to the use of the wet

milling extraction method. The wet milling method uses minimal chemicals compared to most methods and thus preserves DFs (Dalgetty & Baik, 2003). The use of acids, alkalis, alcohols and detergents in non-enzymatic-gravimetric, enzymatic-gravimetric and enzymatic-chemical extraction methods results in the loss of some fibre components resulting in a lower yield (Mwaikambo, 2006).

According to the Foodstuffs, Cosmetics and Disinfectant Act, 1972 (Revised 1 March 2010) a food substance is considered high in fibre if it contains more than 6 g per 100 g dry solids. Therefore, BGN can be said to be high in dietary fibre. BGN black-eye and brown-eye varieties are good yielders, hence farmers could cultivate more of them for fibre extraction. The cultivation of the red variety would not be encouraged as this variety is late maturing and is prone to rotting on site (Anon., 2011)

3.4.2 Hydration properties of BGN dietary fibres

1. *Swelling capacities of BGN insoluble dietary fibres*

The swelling capacity (SWC) of dietary fibres plays a significant role in food systems by acting as a bulking agent, stabiliser, thickener and anti-caking agent (Rosell *et al.*, 2009). The SWC of IDFs ranged from 5.50 mL/g (brown) to 6.50 mL/g (brown-eye) as shown in Table 3.2. There was no significant difference ($p > 0.05$) in the SWCs of brown and red IDFs and also among black-eye, brown-eye and red IDFs. Brown-eye and black-eye had significantly ($p < 0.05$) higher SWCs than red and brown IDFs. Varietal variations could be the cause of variations in SWC among the four BGN IDFs. Similar SWCs in the range 6.50 mL/g (red) to 7.72 mL/g (brown-eye) were reported for BGN IDFs extracted using the enzymatic-gravimetric method (Diedericks, 2014).

Swelling capacities in the range 4.28 mL/g to 5.51 mL/g were reported for chickpea and pea IDF (Dalgetty & Baik, 2003; Huang *et al*., 2009; Elleuch *et al*., 2011). These values are lower than those obtained in this study, hence making BGN IDFs superior in SWC than chickpea and pea IDFs. A swelling capacity of 5.51 mL/g was reported for mung bean hulls sieved through a 50 µm mesh which is similar to the sieve size used in this study (Huang *et al.*, 2009). The similarities in the results of the two studies suggested that particle size plays a major role in determining the SWC of fibres (Huang *et al*., 2009). Increasing SWC with decreasing particle size had been reported (Huang *et al*., 2009). The SWCs of BGN fibres are comparable to that of cellulose (6.2 mL/g) a dietary fibre constituent that is widely used in food products as a bulking agent, stabiliser, thickener and anti-caking agent owing to its hydration properties (Rosell *et al.*, 2009). Reduced cooking losses, decreased firmness, decreased adhesiveness and reduced stickiness in pea and inulin fibre enriched pastas were reported (Tudorica *et al.*, 2002). The researchers reported that the increase in swelling capacities of the fibres imparted these desirable characteristics in the pastas.

Table 3.2 Hydration properties of Bambara groundnut insoluble dietary fibres

Variety	SWC (mL/g)	WHC (g water/g sample)
Black-eye	6.2 ± 0.3^{a}	2.8 ± 0.1^{a}
Brown-eye	6.5 ± 0.0^{a}	2.8 ± 0.1^{a}
Brown	5.5 ± 0.0^{b}	2.6 ± 0.0^{b}
Red	6.0 ± 0.5^{ab}	2.4 ± 0.1^{c}

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different. SWC: Swelling capacity; WHC: Water holding capacity.

This study suggested that BGN fibres would make suitable substitutes for cellulose, inulin and pea fibres in food systems such as pasta due to their SWCs. Physiologically, the SWC of fibres is important in the control of blood glucose levels and also contributes to proper gut function (Hawkes, 2006). As such, BGN dietary fibres would be expected to bind water, swell and slow down digestion, which in turn would slow down carbohydrate absorption, consequently lowering postprandial glucose (Daou & Zhang, 2011). In addition, the SWC of BGN dietary fibres will enable them to increase faecal weight in the human gut thus reducing the risk of constipation (Rosell *et al.*, 2009).

2. *Water holding capacities of BGN dietary fibres*

The water holding capacity (WHC) of BGN IDFs ranged from 2.41 g water/g sample (red) to 2.84 g water/g sample (black-eye) as shown in Table 3.2. The WHCs of black-eye and brown-eye IDFs were significantly ($p < 0.05$) higher than the WHC of brown IDF. Brown IDF, in turn, had a significantly ($p < 0.05$) higher WHC than red IDF. The differences in the WHCs among the four BGN varieties could be due to structural differences as well as variations in composition of the fibres owing to varietal differences (Tosh & Yada, 2010). Diedericks (2014) reported slightly lower WHCs in the range 1.63 g water/g sample (red) to 2.01 g water/g sample (black-eye) for BGN IDFs extracted using the enzymatic-gravimetric method. The lower values could be due to the different methods applied in BGN fibre extraction. Dietary fibre binds water in several ways such as through 1) polar interactions involving anions and cations, 2) ionic interactions, 3) hydrogen bonds, 4) hydrophobic interactions and 5) trapping of water involving capillary action (Chaplin, 2003). The harsh conditions involving repeated enzymatic digestion and chemical (sodium hydroxide, hydrochloric acid and ethanol) treatment BGN fibres were subjected to during the enzymatic-gravimetric extraction could have weakened the fibre structure and disrupted the arrangements of fibre strands (Gordon *et al.*, 2002). This would have resulted in fibres with a reduced ability to bind water therefore explaining the lower WHC

Chau & Huang (2004) reported the WHC of passion fruit seed IDF as 2.4 g water/g sample. Passion fruit seed fibre has been described as a functional ingredient that improves the health and functioning of the gut due to its high WHC (Chau *et al.*, 2005). As the WHC of BGN IDFs is comparable to that of passion fruit seed IDF, it can be deduced that BGN IDFs can play a similar physiological role.

Several researchers reported the WHCs of various legumes (mung bean, adzuki bean, rice bean, Egyptian bean, chickpea, peas and lentils) in the range 3.13 g water/g sample to 13.4 g water/g sample (Dalgetty & Baik 2003; Huang *et al*., 2009; Tiwari & Cummins, 2011; Elleuch *et al*., 2011). The higher values obtained by these researchers could be due to different particle sizes, differences in the chemical nature and composition of the fibres as well as different processing conditions (Tosh & Yada, 2010). The differences

can also be attributed to the difference in legume species. It is expected that dietary fibres of different origin will behave differently and will possess different physicochemical properties.

The WHC of dietary fibres is important as it plays a significant role in increasing satiety of fibre-fortified products, reducing syneresis as well as modifying the viscosity of some food products. Physiologically, WHC is of importance as it positively affects laxation and improves peristalsis (Esposito *et al.*, 2005; Elleuch *et al*., 2011). The WHC of fibres also plays an important role in baked products as more water can be added to the product resulting in better handling and the products stay fresh for longer. In addition, WHC plays an economic role because the more the water is added, the less flour goes into the formulation (Kohajdova *et al.*, 2013).

3.4.3 Densities of BGN dietary fibres

1. *Bulk density*

The bulk densities of BGN fibres were evaluated and the findings are given in Table 3.3. SDFs had bulk densities in the range 0.46 g/mL (black-eye and brown-eye) to 0.57 g/mL (brown). Red SDF was significantly ($p < 0.05$) higher than both brown-eye and black-eye SDFs but lower ($p < 0.05$) than brown SDF. The bulk densities of brown-eye and black-eye SDFs were not significantly ($p > 0.05$) different.

Dalgetty & Baik (2003) reported slightly higher bulk densities for pea, lentil and chickpea SDFs (0.8 g/mL to 0.83 g/mL). Structural differences could be the reason for the differences observed. Diedericks (2014) also reported higher bulk densities for BGN SDFs in the range 0.81 g/mL (brown-eye) to 0.93 g/mL (brown). The lower bulk densities obtained in this study are desirable because the fibres will occupy less packaging space resulting in cost saving. The bulk densities of IDFs ranged between 0.57 g/mL (red and black-eye) to 0.67 g/mL (brown-eye) (Table 3.3). Brown-eye, red and brown IDFs bulk densities differed significantly ($p < 0.05$), however, there was no significant difference ($p > 0.05$) between black-eye and red IDFs and also between black-eye and brown IDFs.

The bulk densities of BGN IDFs were comparable to those of passion fruit seeds (0.68 g/mL), mung bean (0.64 g/mL), green gram (0.69 g/mL), soybean (0.43 g/mL), peas (0.54 – 0.56 g/mL), pigeon pea (0.47 g/mL) and chickpea (0.65 g/mL) IDFs (Dalgetty & Baik, 2003; Chau & Huang, 2004; Huang *et al*., 2009; Maskus, 2010). These fibres are commercially available hence this is one of the indications that BGN has potential to successfully compete with other fibres on the market. The higher bulk densities obtained by Diedericks (2014) could be an indication that the enzymatic-gravimetric method alters the structures of BGN fibres consequently affecting their physical properties.

Table 3.3 Direct and bulk densities of Bambara groundnut fibres

	Bulk density (g/mL)		Direct density (g/mL)	
Variety	**IDF**	**SDF**	**IDF**	**SDF**
Black-eye	0.6 ± 0.0^{ab}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.1 ± 0.0^{a}
Brown-eye	0.7 ± 0.0^{c}	0.5 ± 0.0^{a}	0.5 ± 0.0^{ab}	0.1 ± 0.0^{b}
Brown	0.6 ± 0.0^{a}	0.6 ± 0.0^{b}	0.5 ± 0.0^{c}	0.1 ± 0.0^{c}
Red	0.6 ± 0.0^{b}	0.5 ± 0.0^{c}	0.5 ± 0.0^{b}	0.1 ± 0.0^{d}

Values are mean ± standard deviation. Means within a column followed by the same letter are not significantly [$p > 0.05$] different. IDF: Insoluble dietary fibre. SDF: Soluble dietary fibre.

Dalgetty & Baik (2003) concluded that SDFs have higher densities than IDFs. The results obtained in this study disagree with this conclusion as higher bulk densities were obtained for IDFs than for SDFs with the exception of red SDF that had a higher bulk density (0.57 g/mL) than its IDF counterpart (0.56 g/mL).

2. *Direct density*

The direct densities of four BGN fibres are given in Table 3.3. The direct densities of BGN SDFs ranged from 0.05 g/mL (black-eye) to 0.11 g/mL (brown) and were all significantly ($p < 0.05$) different. Dalgetty & Baik (2003) reported direct densities between 0.47 g/mL and 0.65 g/mL for peas, chickpea and lentils SDFs which are all relatively higher than BGN SDFs. The lower direct densities of BGN SDFs are advantageous because they will pack closer together hence requiring less packaging material, thus reducing packaging costs as well as storage space (Diedericks, 2014).

The direct densities of BGN IDFs ranged from 0.45 g/mL (brown) to 0.53 g/mL (black-eye). The IDFs of black-eye and brown-eye as well as black-eye and red varieties did not differ significantly ($p > 0.05$) in their direct densities. Brown IDF had a significantly lower ($p < 0.05$) direct density than the other three IDFs. Dalgetty & Baik (2003) reported higher direct densities between 0.12 g/mL and 0.17 g/mL for pea, chickpea and lentil IDFs. It was observed that BGN fibres had lower direct densities than most fibres hence had a packaging advantage over these fibres.

3.4.4 The oil binding capacities of BGN dietary fibres

The oil binding capacities (OBCs) of SDFs ranged from 2.78 g oil/g sample (brown) to 4.03 g oil/g sample (brown-eye) as shown in Figure 3.4. Black-eye and red SDFs did not differ significantly ($p > 0.05$) in OBC, brown-eye SDF was significantly ($p < 0.05$) higher than the other three SDFs whilst brown was significantly ($p < 0.05$) lower than all three SDFs. Lower OBCs for pea (1.15 g oil/g sample), chickpea (1.14 g oil/g sample) and lentil (0.89 g oil/g sample) SDFs were reported (Dalgetty & Baik, 2003). Diedericks (2014) reported comparable OBCs for BGN SDFs in the range 4.04 g oil/g sample (black-eye) to 4.55 g oil/g sample.

Among the IDFs, brown IDF had the highest OBC of 1.52 g oil/g sample, brown-eye and black-eye IDFs both had the lowest OBC of 1.40 g oil/g sample. The OBC of all four IDFs did not differ significantly ($p > 0.05$). BGN IDFs had lower OBCs compared to SDFs. Higher IDF OBC values were reported for peas (6.93 g oil/g sample), chickpea (4.25 g oil/g sample) and lentils (4.01 g oil/g sample) by Dalgetty & Baik (2003). These differences can be attributed to the different structures of the fibres, species variation, different particle sizes as well as the variation in their composition which would determine their oil binding capabilities (Dhingra *et al.*, 2012).

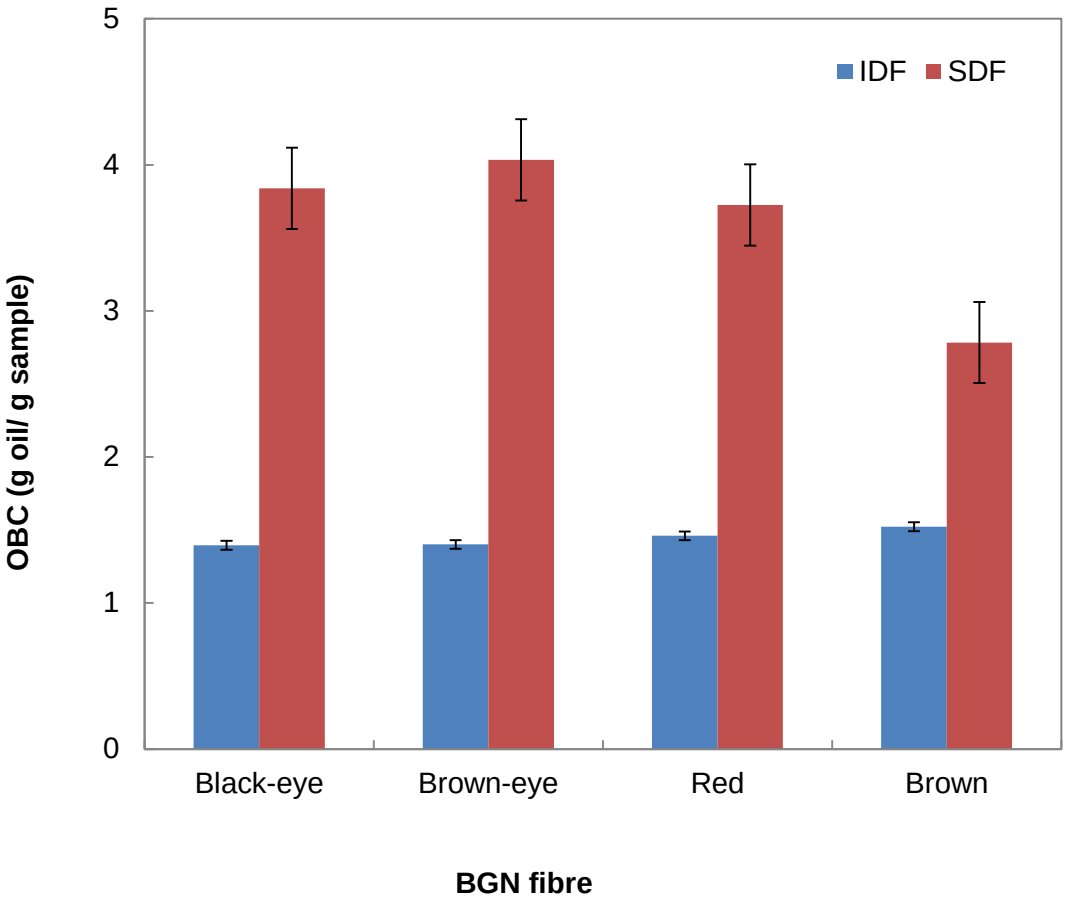

Figure 3.4 The oil binding capacity (OBC) of Bambara groundnut (BGN) insoluble and soluble dietary fibres (IDF and SDF, respectively).

OBCs similar to those obtained in this study were reported for mung bean hulls (1.49 g oil/g sample to 1.83 g oil/g sample) and BGN IDFs (1.38 g oil/g sample – 1.52 g oil/g sample) (Huang *et al*., 2009; Diedericks, 2004).

Rosell *et al.* (2009) studied eleven commercial fibres and reported low OBC values with the highest being 0.02 g oil/g sample from bamboo. This indicated that BGN fibres can compete commercially with other fibres for their OBCs in stabilising high fat food products and emulsions as well as act as fat binders and replacers in meat and meat products (Elleuch *et al.*, 2011). This study indicated that the use of BGN fibres would be economical as less BGN fibre would be used to render the desirable properties compared to other leguminous fibres. The ability of BGN fibres to bind oil can be harnessed by the food industry to reduce fat losses upon cooking and stabilising emulsions (Elleuch *et al.*, 2011; Slavin, 2013) Physiologically, the OBC of BGN fibres would allow them to play a role in bile acid absorption and consequently cholesterol reduction (Tosh & Yada, 2010).

3.4.5 Colour characteristics of BGN dietary fibres

The colour attributes of BGN measured were lightness (L*), greenness (-a*), redness (+a*), blueness (-b*), yellowness (+b*), hue and chroma (Table 3.4). Lightness is the luminous intensity of colour measured on a scale of 0 to 100, with 0 indicating black and 100 indicating white. Hue is how the colour of an object is perceived and chroma is the vividness or dullness of colour, it indicates the richness or saturation of a colour (Manera *et al*., 2012; Murevanhema, 2012). On the colour scale, a* describes the redness or the greenness of a colour with positive a* indicating redness and negative a* indicating greenness and 0 being neutral.

The lightness of BGN SDFs ranged from 70.96 (red) to 74.04 (brown-eye) as shown in Table 3.4. There was no significant ($p > 0.05$) difference in lightness between black-eye and brown-eye, black-eye and brown as well as between brown and red SDFs. The redness (a*) values of SDFs were in the range 1.66 (black-eye) to 2.54 (red). There was no significant difference among the red, brown and black-eye SDFs. The yellowness (b*) of SDFs ranged from 13.82 (black-eye) to 15.56 (red) (Table 3.4).

Black-eye SDF was significantly ($p < 0.05$) lower in yellowness than the other three SDFs. The chroma of SDFs ranged from 13.91 (black-eye) to 16.29 (red). There was no significant ($p > 0.05$) difference in redness among the red, brown and black-eye SDFs. The hue angle ranged from 79.81° (red) to 83.13° (black-eye) for the SDFs. There was no significant ($p > 0.05$) difference in hue among the black-eye, brown-eye and brown SDFs as well as among brown-eye, brown and red SDFs. SDFs were lighter in colour and had higher hues than their insoluble counterparts. Red SDF was characterised by high redness, yellowness and chroma but had the least saturation and hue. Black-eye and brown-eye fibres were relatively lighter than the brown and red fibres.

Table 3.4 Colour attributes of Bambara groundnut dietary fibres

	L*	a*	b*	Chroma	Hue (°)
IDF					
Black-eye	36.6 ± 0.4^{a}	9.9 ± 0.1^{a}	17.6 ± 0.3^{a}	20.2 ± 0.3^{a}	60.6 ±0.3^{a}
Brown-eye	37.9 ± 0.5^{b}	10.1 ± 0.1^{a}	18.5 ± 0.2^{b}	21.1 ± 0.2^{a}	61.5 ±0.2^{b}
Brown	24.3 ± 0.1^{c}	6.0 ± 0.1^{b}	7.9 ± 0.1^{c}	10.0 ± 0.1^{b}	52.8 ±0.4^{c}
Red	30.8 ± 0.2^{d}	7.9 ± 0.1^{c}	10.5 ± 0.2^{d}	12.7 ± 0.9^{c}	52.8 ±0.1^{c}
SDF					
Black-eye	73.0 ± 0.2^{ab}	1.7 ± 0.0^{a}	13.8 ± 0.1^{a}	13.9 ± 0.1^{a}	83.1 ±0.1^{a}
Brown-eye	74.0 ± 0.5^{a}	2.4 ± 0.0^{b}	15.5 ± 0.4^{b}	15.7 ± 0.1^{b}	81.2 ±0.5^{ab}
Brown	71.7 ± 1.1^{bc}	2.3 ± 0.3^{b}	15.5 ± 0.3^{b}	15.9 ± 0.8^{b}	81.1 ±1.8^{ab}
Red	71.0 ± 1.3^{c}	2.5 ± 0.2^{b}	15.6 ± 0.5^{b}	16.3 ± 1.0^{b}	79.8 ±1.4^{b}

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different;
L*: lightness; a*: red/ green; b*: yellow/blue; IDF: insoluble dietary fibre; SDF: soluble dietary fibre.

The lightness of BGN IDFs ranged from 24.30 (brown) to 37.85 (brown-eye) (Table 3.4). All four IDFs differed significantly ($p < 0.05$) in lightness. The redness (a*) values of IDFs were in the range 6.03 (brown) to 10.05 (brown-eye). There was no significant ($p > 0.05$) difference in redness between black-eye and brown-eye IDFs. The yellowness (b*) of IDFs was in the range 7.91 (brown) to 18.52 (brown-eye) and all four IDFs differed significantly ($p < 0.05$) in yellowness. The chroma of IDFs ranged from 10.00 (brown) to 21.10 (brown-eye) and there was no significant ($p > 0.05$) difference in redness between black-eye and brown-eye IDFs. The hue angle ranged from 52.79° (red) to 61.47° (brown-eye) for the IDFs indicating a yellowish-red colour. There was no difference ($p > 0.05$) in hue between the red and brown fibres. Black-eye and brown-eye IDFs were lighter, redder, yellower, more saturated and had a higher hue compared to the red and brown IDFs. The relatively higher degree of lightness observed in black-eye and brown-eye IDFs can be attributed to their cream coats as the pigments in the red and brown coats affected the colour of the fibres making them duller (Murevanhema, 2012). This observation was supported by Nti (2009) who reported a reduction in lightness of BGN milk with increase in the pigment component in the BGN seed coat. Hence, fibres from cream coated seeds (brown-eye and black-eye) were lighter than those from more heavily pigmented seeds (red and brown).

All the BGN fibres had +a* and +b* values indicating that they were more associated with redness and yellowness, respectively. The redness and yellowness of BGN fibres relate to phenolics and other such chemicals that may possess antioxidant properties. Phenolic compounds associated with redness and yellowness include flavonoids such as quercetin, anthocyanins, isorhamnetin, cyanidins and pelargonidin, isoflavones such as daidzein, genistein and coumestrol as well as phenolic acids such as salicylic acids (Delgado-Vargas *et al.*, 2000; Cheynier, 2005; Dai & Mumper, 2010; Dragovic-Uzelac *et al.*, 2010; Hu & Xu, 2011; Walter & Marchesan, 2011). Lightness of fibres as well as the overall colour of BGN fibres is of importance in food products as it determines the extent to which the original colour of the food will be affected (Rosell *et al.*, 2009; Tosh & Yada, 2010). It was observed that redness increased with increasing yellowness and this was in agreement with Murevanhema (2012). Lightness increased with increasing hue. The varying colours of the BGN dietary fibres are advantageous as the manufacturer will have a choice of a fibre that best suits the colour of their product.

Colour differences among the BGN dietary fibres

A colour difference (ΔE) of 1 is the threshold at which a trained observer would notice the difference between two colours (Sharma, 2005). It is known as a just-noticeable difference (JND). The difference between two colours can be perceivable but still deemed acceptable. A ΔE between 4 and 8 is acceptable and above 8 is unacceptable and likely to be rejected by consumers (Sharma, 2005). Table 3.5 gives the colour difference between BGN fibres.

Table 3.5 Colour differences between Bambara groundnut fibres

Variety	IDF (ΔE)	SDF (ΔE)
Black-eye – Brown-eye	1.55	2.12
Black-eye - Brown	16.14	2.22
Black-eye – Red	18.66	2.81
Brown-eye – Brown	17.67	2.31
Brown-eye – Red	10.94	3.08
Brown – Red	7.20	0.81

ΔE: Colour difference; IDF: insoluble dietary fibre; SDF: soluble dietary fibre

All the SDFs showed acceptable colour differences with ΔE ranging between 0.81 and 3.08 meaning they could be used interchangeably in products without a noticeable difference. Brown and red SDFs had ΔE of 0.81 which is below the threshold therefore the colour difference would not be perceivable. Black-eye – brown, black-eye – red, brown-eye – brown and brown-eye – red all had ΔE above 1 meaning the consumer could perceive the difference but the values are below 8 thus making the difference acceptable. IDFs with the exception of black-eye – brown-eye and brown – red comparisons had ΔE above 8 meaning their colour differences were very apparent and if used interchangeably, a perceivable difference would be expected. Brown and red IDFs as well as brown-eye and black-eye IDFs had acceptable colour differences. This can be attributed to the seed coats of BGN seeds, with brown and red varieties having darker, more saturated seed coats while black-eye and brown-eye varieties have cream coloured seed coats. Insoluble dietary fibre is largely concentrated in the hulls of seeds. Hence, the seed coat colour of BGN seeds may have affected the colours of the resultant IDFs.

3.4.6 Polyphenolic compounds in BGN dietary fibres

1. *Hydrolysable polyphenolic content of BGN fibres*

The HPP content of SDFs ranged from 6.89 mg/g GAE (brown) to 20.86 mg/g GAE (brown-eye) as shown in Figure 3.5. All four SDFs differed significantly ($p < 0.05$) in their HPP content. Diedericks (2014) reported relatively higher HPP contents of BGN SDFs in the range 45.42 mg/g GAE (brown-eye) to 55.90 mg/g GAE (black-eye). Since same BGN varieties were investigated the most probable explanation for differing HPP contents could be attributed to the different extraction methods used. The enzymatic-gravimetric method could have a positive effect on the availability of HPPs in BGN SDFs. IDFs had an HPP content ranging from 10.96 mg/g GAE (black-eye) to 14.43 mg/g GAE (brown). Black-eye and brown-eye as well as brown and red IDFs did not differ significantly ($p > 0.05$) in their HPP content. Similar HPPs were reported for BGN IDFs in the range 6.14 mg/g GAE (brown-eye) to 15.56 mg/g GAE (brown) (Diedericks, 2014).

The high polyphenolic composition of BGN fibres revealed their potential antioxidant properties. Elleuch *et al*. (2011) suggested that fibres can be exploited as potential novel antioxidants and would be of importance in protecting against superoxide radicals, hydroxyl free radicals and lipid peroxidation. BGN fibres would thus find use as ingredients in fatty foodstuffs to improve oxidative stability and hence extending their shelf life (Elleuch *et al*., 2011). They would also enhance the antioxidant properties of whichever product they are used in, therefore could be considered as fortifying agents. Antioxidants are important for human health as they mitigate the risk of degenerative diseases like cancers and decrease the oxidation of low density lipoproteins thereby avoiding arteriosclerosis and related coronary heart diseases (Tomas *et al.*, 2004; Tsimikas, 2006).

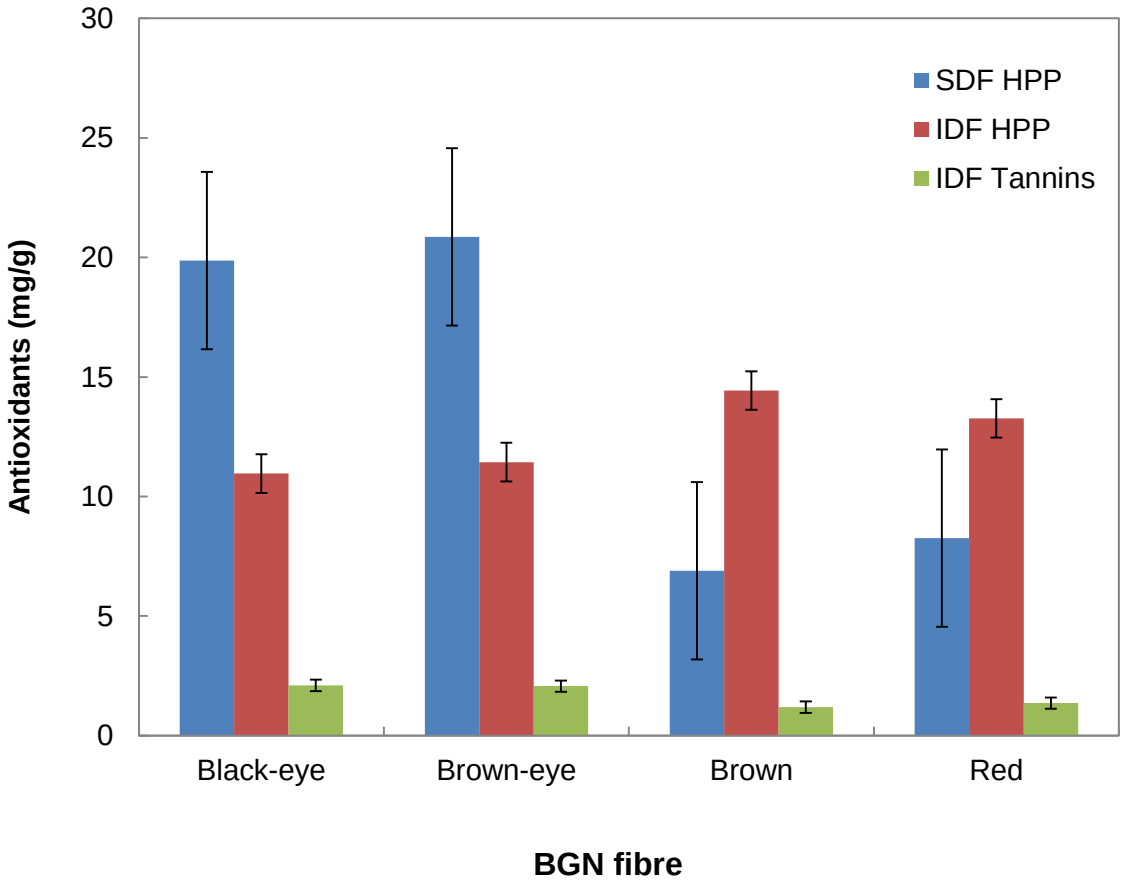

Figure 3.5 The hydrolysable polyphenols (HPP) and tannin content of Bambara groundnut (BGN) insoluble and soluble dietary fibres (IDF and SDF, respectively).

Antioxidants carry out these functions by reacting with free radicals forming stable or non-reactive radicals (Betancur-Ancona *et al.*, 2004). BGN fibres can be a useful source of natural antioxidants as artificial antioxidants have been reported to be carcinogenic and teratogenic (Betancur-Ancona *et al.*, 2004).

2. *Condensed tannins*

Brown IDF had the least tannin content of 1.1 mg/g and black-eye IDF had the highest tannin content (2.1 mg/g). There was no significant ($p > 0.05$) difference between black-eye and brown-eye IDFs, both were significantly higher ($p < 0.05$) than red and brown IDFs in tannin content. Brown IDF was significantly ($p < 0.05$) lower than the other three fibres. The difference among the fibres is in agreement with Nti (2009) who reported that chemical composition and tannin content differs from one BGN variety to another. Diedericks (2014) also reported low tannin contents for BGN IDFs below 1 mg. The low tannin content observed in BGN fibres could be advantageous as tannins have been associated with anti-nutritional properties due to their ability to form complexes with some nutrients, including divalent minerals and proteins rendering them bio-unavailable (Saura-Calixto & Bravo, 2001).

3.4.7 Neutral sugars and uronic acid content of BGN dietary fibres

1. *Neutral sugars*

Seven neutral sugars were analysed for in BGN fibres and the results are given in Table 3.6. Arabinose and galactose coeluted, therefore are presented as arabinose/galactose. The percentage of arabinose/galactose in SDFs was in the range 9.4% (brown) to 19.6% (black-eye). The coelution of arabinose and galactose can be explained by the findings of Pfoertner & Fischer (2001) who described that some fibres consist of a rhamnogalacturonan backbone with galactose and arabinose containing side chains. Therefore, the co-elution of arabinose and galactose could be indicative of the presence of rhamnogalacturonan. The percentage of fructose in SDFs ranged from 1.3% (black-eye) to 1.7% (brown).

Fucose and glucose were obtained in low amounts (below 1%) in SDFs while relatively higher percentages of xylose were obtained in the range 13.0% (brown) to 16.6% (black-eye) (Table 3.6). Brown-eye and black-eye SDFs did not differ significantly ($p > 0.05$) in their sugar composition with the exception of arabinose/galactose. Rhamnose was absent in all SDFs. This is in agreement with Dalgetty & Baik (2003) who reported the absence of rhamnose in SDFs of pea, lentil and chickpea. The researchers also reported the absence of arabinose and mannose in SDFs. In this study however, these two sugars were identified. The presence of these sugars in BGN SDFs suggests the possible presence of galactomannans, arabinoxylan and arabinogalactans.

Table 3.6 Neutral sugar composition of Bambara groundnut dietary fibres

	SUGARS (%)							
Variety	**Arabinose/ galactose**	**Fructose**	**Fucose**	**Glucose**	**Mannose**	**Rhamnose**	**Xylose**	**Total sugar**
SDF								
Black-eye	19.6 ± 0.9^{a}	1.3 ± 0.1^{a}	0.3 ± 0.1^{a}	1.0 ± 0.1^{a}	6.1 ± 0.5^{a}	0	16.6 ± 1.6^{a}	44.8
Brown-eye	15.2 ± 1.5^{b}	1.5 ± 0.1^{ab}	0.3 ± 0.1^{a}	0.8 ± 0.1^{ab}	6.5 ± 0.2^{a}	0	15.6 ± 0.2^{ab}	39.8
Brown	9.4 ± 0.1^{c}	1.7 ± 0.1^{b}	0.1 ± 0.0^{b}	0.8 ± 0.1^{ab}	7.4 ± 0.6^{b}	0	13.0 ± 1.8^{c}	32.5
Red	10.0 ± 0.6^{c}	1.6 ± 0.1^{b}	0.3 ± 0.0^{a}	0.6 ± 0.2^{b}	5.0 ± 0.3^{c}	0	13.7 ± 0.8^{bc}	31.2
IDF								
Black-eye	2.8 ± 0.2^{a}	1.7 ± 0.1^{ac}	0.1 ± 0.0^{a}	0.6 ± 0.1 ^{a}	4.7 ± 0.4^{a}	2.6 ± 0.1^{a}	11.3 ± 1.2^{a}	23.8
Brown-eye	2.3 ± 0.1^{b}	1.7 ± 0.1^{a}	0.3 ± 0.1^{b}	0.7 ± 0.1^{ab}	6.6 ± 0.1^{ab}	1.0 ± 0.1^{b}	11.3 ± 1.9^{a}	23.9
Brown	2.5 ± 0.2^{ab}	1.5 ± 0.1^{b}	0.4 ± 0.1^{b}	0.9 ± 0.1^{c}	5.6 ± 0.4^{c}	1.2 ± 0.1^{c}	13.8 ± 2.3^{a}	25.8
Red	2.8 ± 0.2^{a}	1.9 ± 0.1^{c}	0.2 ± 0.1^{a}	0.8 ± 0.0^{b}	5.6 ± 0.6^{b}	1.1 ± 0.1^{c}	12.4 ± 0.8^{a}	24.7

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different. IDF: insoluble dietary fibre; SDF: soluble dietary fibre.

Galactomannans are related to locust bean and guar gums, their solubility in water increases with increasing galactose content. BGN SDFs had higher quantities of galactose compared to mannose. Silveria & Bresolin (2011) reported an increase in the solubility of galactomannans with increasing quantities of galactose and decreasing quantities of mannose. Galactomannans in BGN SDFs would be expected to have a high solubility. Arabinoxylans possess antioxidant capabilities and influence water balance and rheology and arabinogalactans possess similar characteristics as gum Arabic (Golenser *et al.*, 1999; Sivam *et al.*, 2010; Saeed *et al.*, 2011; Khotimchenko *et al.*, 2012). The suggestive presence of these hydrocolloids in BGN fibres could mean that BGN fibres possess similar beneficial characteristics and thus can be classified with them. The presence of rhamnose and galactose in BGN IDFs suggests the presence of some polysaccharides such as pectic substances which in turn suggests the presence of rhamnogalacturonans (Huang *et al.*, 2009; Diedericks, 2014). Rhamnogalacturonans are reported to bind heavy metals in the human body as well as lower blood cholesterol (Sivam *et al.*, 2010; Khotimchenko *et al.*, 2012). The absence of arabinose and mannose in pea, lentil and chickpea SDFs reported by Dalgetty & Baik (2003) was indicative of the absence of galactomannans, arabinoxylan and arabinogalactans in those fibres hence making BGN IDFs more superior in carbohydrate composition.

In IDFs, arabinose/galactose was in the range 2.3% (brown-eye) to 2.8% (red). The percentage of fructose in IDFs ranged from 1.5% (brown) to 1.9% (red). Fucose and glucose were obtained in low amounts (below 1%) while rhamnose ranged from 1.0% (brown-eye) to 2.6% (black-eye). Relatively high percentages of xylose were obtained in IDFs (Table 3.6). The presence of these in BGN IDFs is an indication of the presence of some polysaccharides such as cellulose (glucose) and hemicellulose (xylose, glucose, arabinose, galactose and mannose) (Dhingra *et al.*, 2012; Diedericks, 2014). The presence of arabinose and xylose in IDFs suggested the presence of arabinoxylans. Low quantities of arabinose/galactose in IDFs suggested low quantities of arabinogalactans.

2. *Uronic acids*

The uronic acid content of SDFs did not differ significantly ($p > 0.05$) and ranged from 10.6% (brown) to 11.5% (red) as shown in Figure 3.6. Dalgetty & Baik (2003) reported lower uronic acids in pea, lentil and chickpea SDFs in the range 0.2 to 1.3%. The uronic acid content of IDFs ranged from 6.7% (black-eye) to 10.6% (red) (Figure 3.6). There was no significant ($p > 0.05$) difference among the red, brown and black-eye IDFs as well as between brown and brown-eye IDFs in uronic acid content. Dalgetty & Baik (2003) reported lower uronic acid of pea, lentil and chickpea IDFs in the range 2.0 - 2.8% indicating the superiority in uronic acid content of BGN IDFs over other leguminous IDFs.

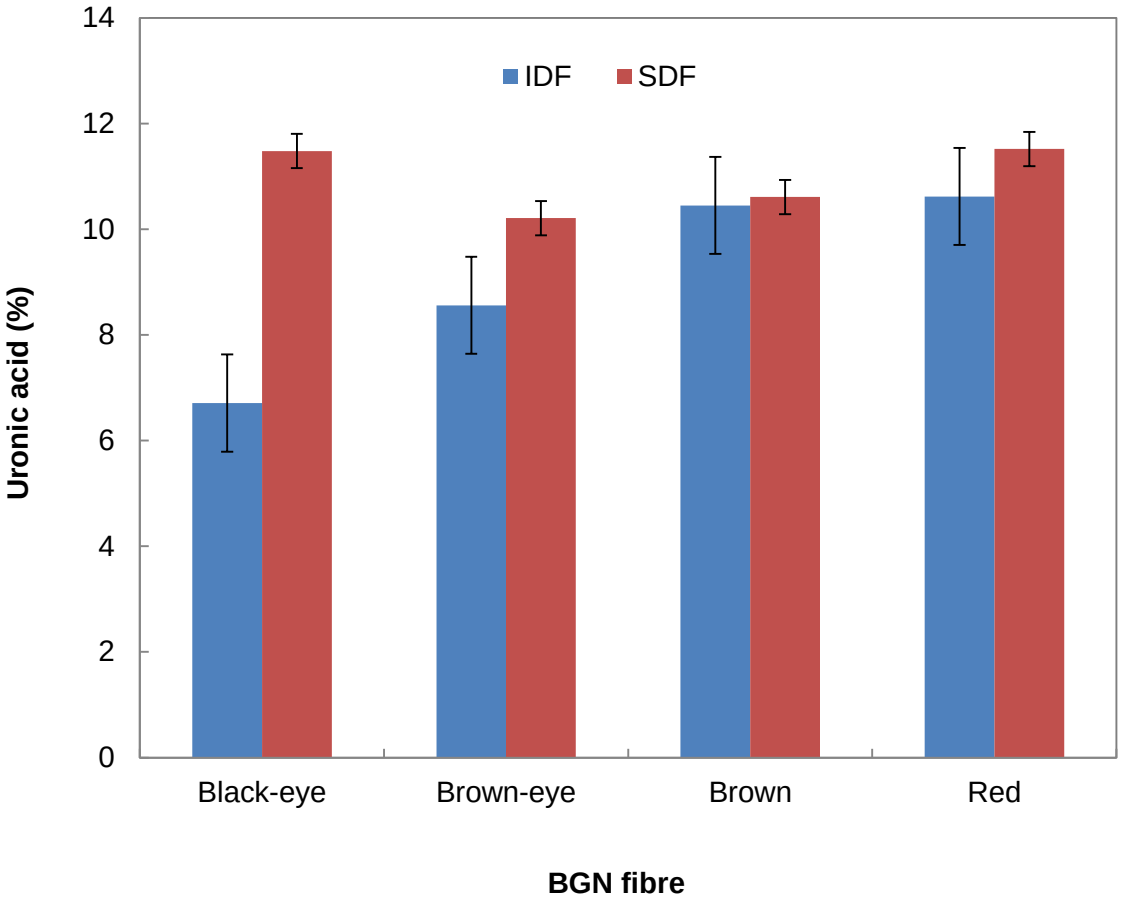

Figure 3.6 Uronic acid composition of Bambara groundnut soluble and insoluble dietary fibres (SDF and IDF, respectively).

SDFs showed a relatively higher amount of uronic acids compared to IDFs which is contradictory to the study of Dalgetty & Baik (2003) who reported higher uronic acid in IDFs than in SDFs of peas, lentils and chickpeas. The differences in these studies could be attributed to the different fibre sources.

Uronic acids are sugar acids with both carbonyl (C = O) and carboxylic acid (COOH) functional groups (Wadouachi & Kovensky, 2011). The presence of high quantities of uronic acids in BGN dietary fibres is of importance because these sugar acids play significant roles in the human body. Uronic acids form salts with some wastes in the human body thereby facilitating their excretion (Vazquez *et al.*, 2013). Furthermore, uronic acids react with less polar compounds such as steroids, bilirubin and drugs increasing their polarity thereby aiding their removal through urine and bile, contributing to the body's detoxification process (Chhabra, 2012). Uronic acids are also responsible for the formation of mucopolysaccharides such as hyaluronic acid and heparin which are major components of mucus, synovial fluid, cornea, cartilage and tendons (Chhabra, 2012). The presence of uronic acids in BGN dietary fibres is also indicative of the presence of pectin. Pectin is a thickening heteropolysaccharide and would be expected to increase the viscosity of food in the stomach, slowing down digestion and thus prolonging satiety. Furthermore, BGN dietary fibres could find use in food systems as thickeners by increasing the viscosity of food systems (Saha & Bhattacharya, 2010).

3.4.8 Rheological properties of BGN soluble fibres

1. *Time dependent rheological properties of BGN soluble dietary fibres*

Time dependent flow properties of a system or structure reflect viscoelasticity, structural changes, or both (Adeyi, 2014). The hysteresis loop area gives an indication of the structural breakdown with higher values indicating more extensive damage (Adeyi, 2014). Table 3.7 gives the extent of the hysteresis loop areas of the four BGN SDFs at different concentrations. The hysteresis loop areas of black-eye SDF were in the range 4.76 Pas^{-1} (14%) to 5.77 Pas^{-1} (6%) and those of brown-eye SDF were in the range 3.46 Pas^{-1} (8%) to 6.78 Pas^{-1} (4%). Brown SDF had hysteresis loop areas in the range 4.89 Pas^{-1} (4%) to 7.03 Pas^{-1} (14%) and the hysteresis loop areas of red SDF were in the range 5.42 Pas^{-1} (4%) to 13.51 Pas^{-1} (8%). The presence of hysteresis loop areas was an indication that all the BGN SDFs were time dependent in nature (Adeyi, 2014). Red SDF showed the least stability at all concentrations as shown by the higher hysteresis loop areas (Table 3.7). However, 4% red BGN SDF showed less structural damage than 4% brown-eye SDF.

The backward sweep not lying directly on top of the forward sweep (Figures 3.7 and 3.8) indicated that the structure of BGN SDFs was destroyed by shearing with time.

Table 3.7 Hysteresis loop areas for different concentrations of Bambara groundnut soluble dietary fibres

BGN SDF concentration (%)	Integrating area for upward curve	Integrating area for downward curve	Hysteresis loop area (Pas^{-1})
Black-eye			
4	38.64	33.53	5.12 ± 0.01
6	43.52	37.75	5.77 ± 0.26
8	47.71	42.17	5.54 ± 0.21
10	50.82	46.04	4.78 ± 0.17
12	55.19	50.21	4.98 ± 0.01
14	61.53	56.77	4.76 ± 0.06
Brown-eye			
4	46.37	39.59	6.78 ± 0.03
6	52.40	48.12	4.28 ± 0.03
8	62.43	58.97	3.46 ± 0.00
10	71.71	66.76	4.95 ± 0.02
12	75.91	70.84	5.07 ± 0.05
14	88.53	82.18	6.35 ± 0.00
Brown			
4	38.69	33.80	4.89 ± 0.00
6	43.70	38.34	5.36 ± 0.00
8	49.22	42.77	6.45 ± 0.00
10	51.99	46.41	5.58 ± 0.07
12	56.40	51.01	5.39 ± 0.01
14	64.20	57.17	7.03 ± 0.02
Red			
4	36.67	31.25	5.42 ± 0.00
6	41.91	35.39	6.52 ± 0.01
8	52.52	39.01	13.51 ± 0.05
10	53.12	42.95	10.17 ± 0.00
12	54.10	47.67	6.43 ± 0.04
14	60.60	47.11	13.49 ± 0.01

Values are mean ± standard deviation. BGN: Bambara groundnut. SDF: soluble dietary fibres.

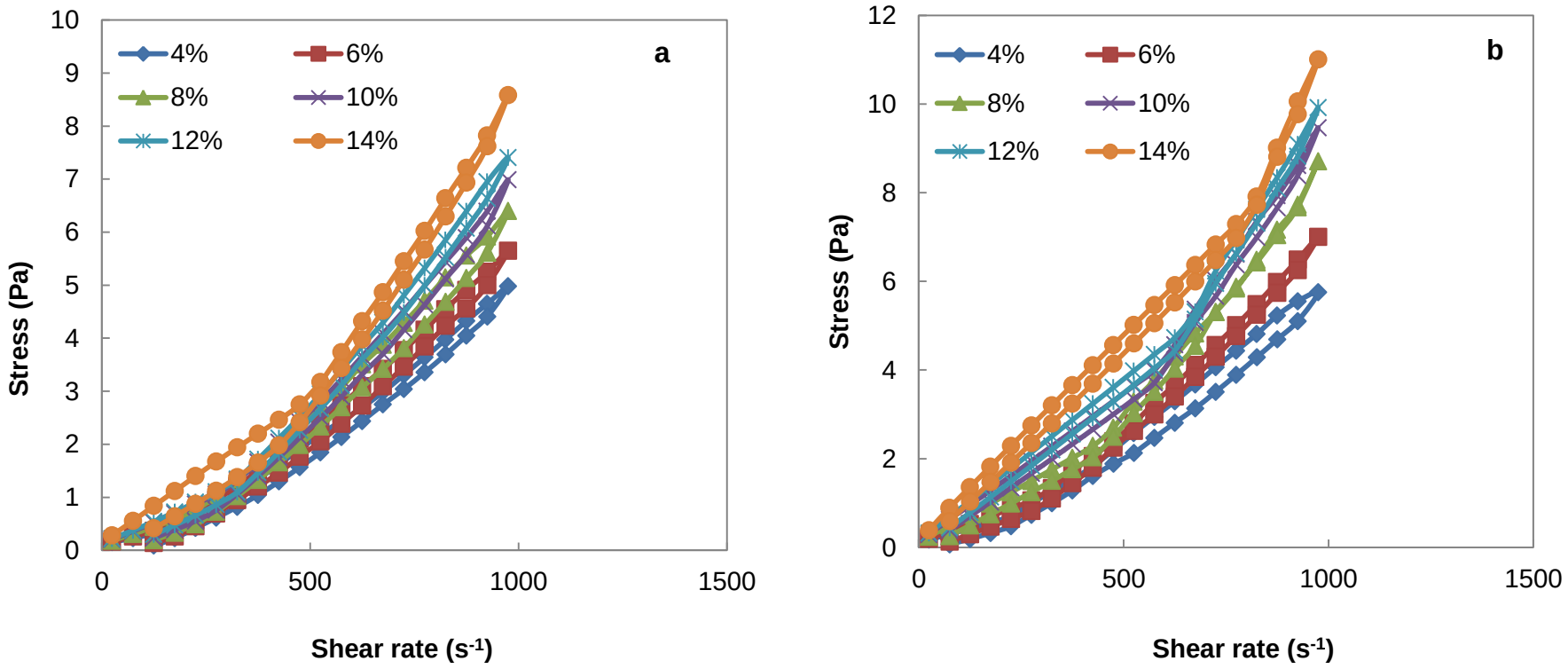

Figure 3.7 Time dependent properties of Bambara groundnut soluble dietary fibres (a) Black-eye (b) Brown-eye.

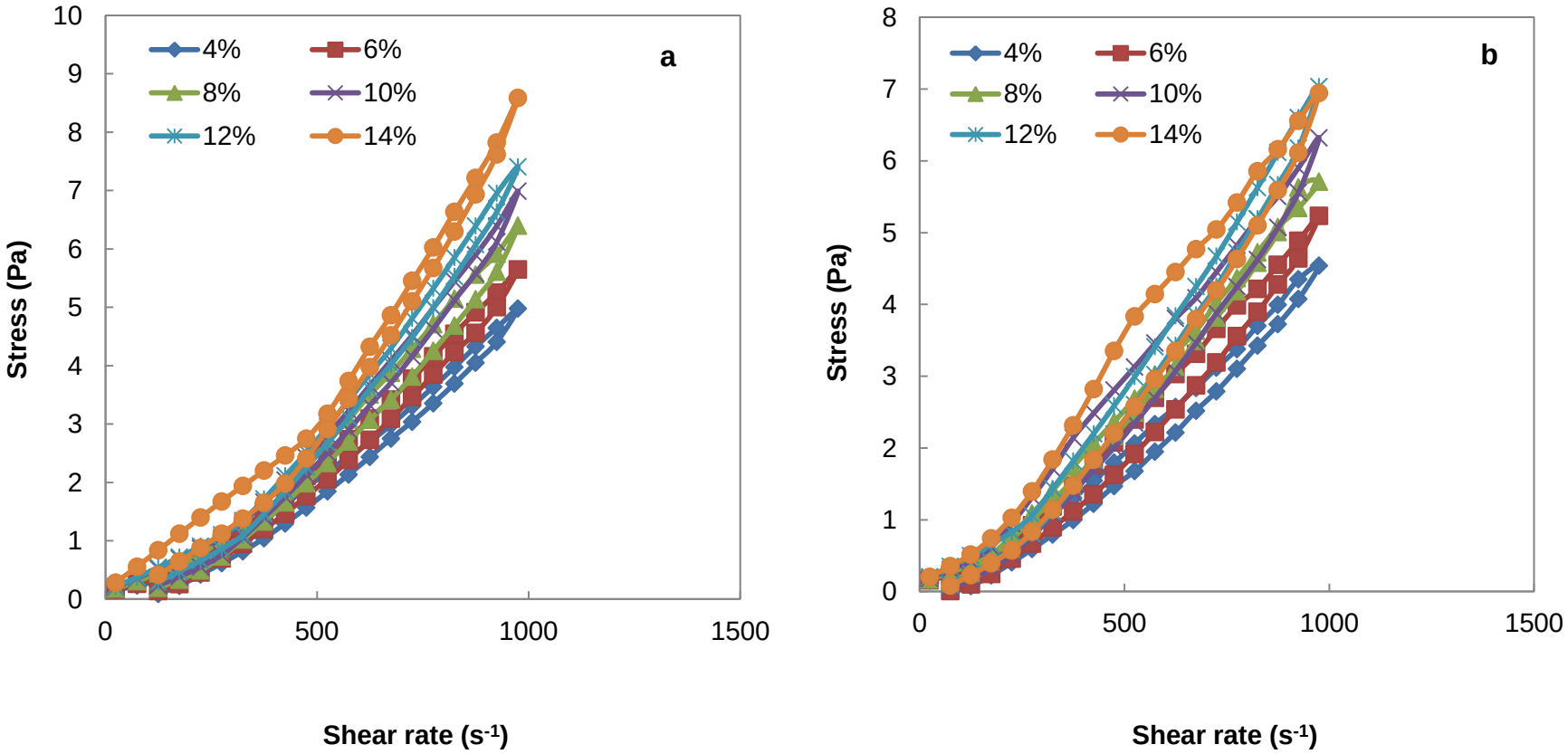

Figure 3.8 Time dependent properties of Bambara groundnut soluble dietary fibres (a) Brown (b) Red.

Black-eye SDF showed more structural disruption at lower concentrations (4 - 8%) compared to higher concentrations (10 - 14%). This indicated that with increase in concentration, the stability of black-eye SDF increased. It was observed that at 14%, red was not the strongest system with 12% exhibiting more stability at higher shear rate (Figure 3.8b). The rheograms indicated that higher concentrations (12% and 14%) exhibited the highest stability, despite the breakdown in structure. Thus, if they were incorporated into a food system they would maintain their structure better than lower concentrations. To preserve the structure and consequently the viscosity of the BGN SDFs in food systems, time of shearing and shear rate could be minimised.

The hysteresis loop area could serve as a tool for the formulator in the food industry. It would give the formulator/manufacturer knowledge of how the structure of the dietary fibres would change with increased shear over prolonged periods of time (Adeyi, 2014). Higher values mean that if the particular dietary fibre is incorporated into systems such as emulsions, they would disintegrate and lose their properties thus contributing to the destabilisation of the whole system.

2. *Time independent rheological properties of BGN soluble dietary fibres*

Power law was used to describe the flow behaviour of the BGN SDFs. Power law consists of two parameters, namely consistency coefficient (K) which describes the viscosity of a system with higher values indicating more viscous solutions and flow behaviour index (n) which is a measure of rigidity or reluctance of a fluid to flow and describes shear rate against shear stress (Adeyi, 2014). Table 3.8 shows the Power law model parameters for the four BGN SDFs. The coefficient of determination (R^2) of all the four SDFs was ≥ 0.95 which indicated that Power law could be accurately employed for predicting the intrinsic rheological properties of the fibres (Gomez-Romero *et al.*, 2014). The consistency coefficient (K) increased with increasing concentration for all SDFs. The consistency coefficients (K) of 4-14% concentrations of black-eye, brown-eye, red and brown SDFs were 0.0024 $Pa{\cdot}s^n$ - 0.0055 $Pa{\cdot}s^n$, 0.0031 $Pa{\cdot}s^n$ - 0.0192 $Pa{\cdot}s^n$, 0.002 $Pa{\cdot}s^n$ (4%) - 0.0091 $Pa{\cdot}s^n$ and 0.0026 $Pa{\cdot}s^n$ - 0.0037 $Pa{\cdot}s^n$, respectively. Red SDF showed the least viscosity and brown-eye SDF showed the highest viscosity. The flow behaviour index (n) was observed to decrease with increasing concentration as an indication of the reluctance to flow of the system.

Higher concentrations of BGN SDFs had higher viscosities as displayed by Figures 3.9 and 3.10. Red SDF had the least viscosity, with 14% having an initial apparent viscosity of 8.5^{-3} Pas^{-1} while brown-eye SDF had the highest initial viscosity, with 14% having an initial apparent viscosity of 1.6^{-2} Pas^{-1}.

Table 3.8 Power law model parameters for four Bambara groundnut soluble dietary fibres

Concentration (%)	K ($Pa \cdot s^n$)	n	R^2
Black-eye			
4	0.0024 ± 0.00	1.0794 ± 0.00	0.9524
6	0.0027 ± 0.00	1.0787 ± 0.00	0.9501
8	0.0031 ± 0.00	1.0700 ± 0.01	0.9479
10	0.0034 ± 0.00	1.0676 ± 0.00	0.9469
12	0.0042 ± 0.00	1.0490 ± 0.00	0.9451
14	0.0055 ± 0.00	1.0241 ± 0.00	0.9540
Brown-eye			
4	0.0031 ± 0.00	1.0695 ± 0.00	0.9518
6	0.0039 ± 0.00	1.0529 ± 0.00	0.9546
8	0.0064 ± 0.00	1.0044 ± 0.00	0.9662
10	0.0103 ± 0.00	0.9541 ± 0.00	0.9779
12	0.0123 ± 0.00	0.9359 ± 0.00	0.9816
14	0.0192 ± 0.01	0.8926 ± 0.00	0.9940
Brown			
4	0.0025 ± 0.00	1.9502 ± 0.00	0.9516
6	0.0027 ± 0.00	1.0190 ± 0.00	0.9525
8	0.0031 ± 0.00	1.0401 ± 0.00	0.9529
10	0.0042 ± 0.00	1.0763 ± 0.00	0.9505
12	0.0052 ± 0.00	1.0848 ± 0.00	0.9551
14	0.0091 ± 0.00	1.0791 ± 0.00	0.9665
Red			
4	0.0026 ± 0.00	1.0618 ± 0.02	0.9578
6	0.0025 ± 0.00	1.0916 ± 0.00	0.9523
8	0.0026 ± 0.00	1.1015 ± 0.00	0.9588
10	0.0033 ± 0.00	1.0866 ± 0.00	0.9649
12	0.0035 ± 0.00	1.0596 ± 0.00	0.9495
14	0.0037 ± 0.00	1.0087 ± 0.00	0.9664

Values are mean ± standard deviation. K: consistency coefficient; n: flow behaviour index; R^2; coefficient of determination.

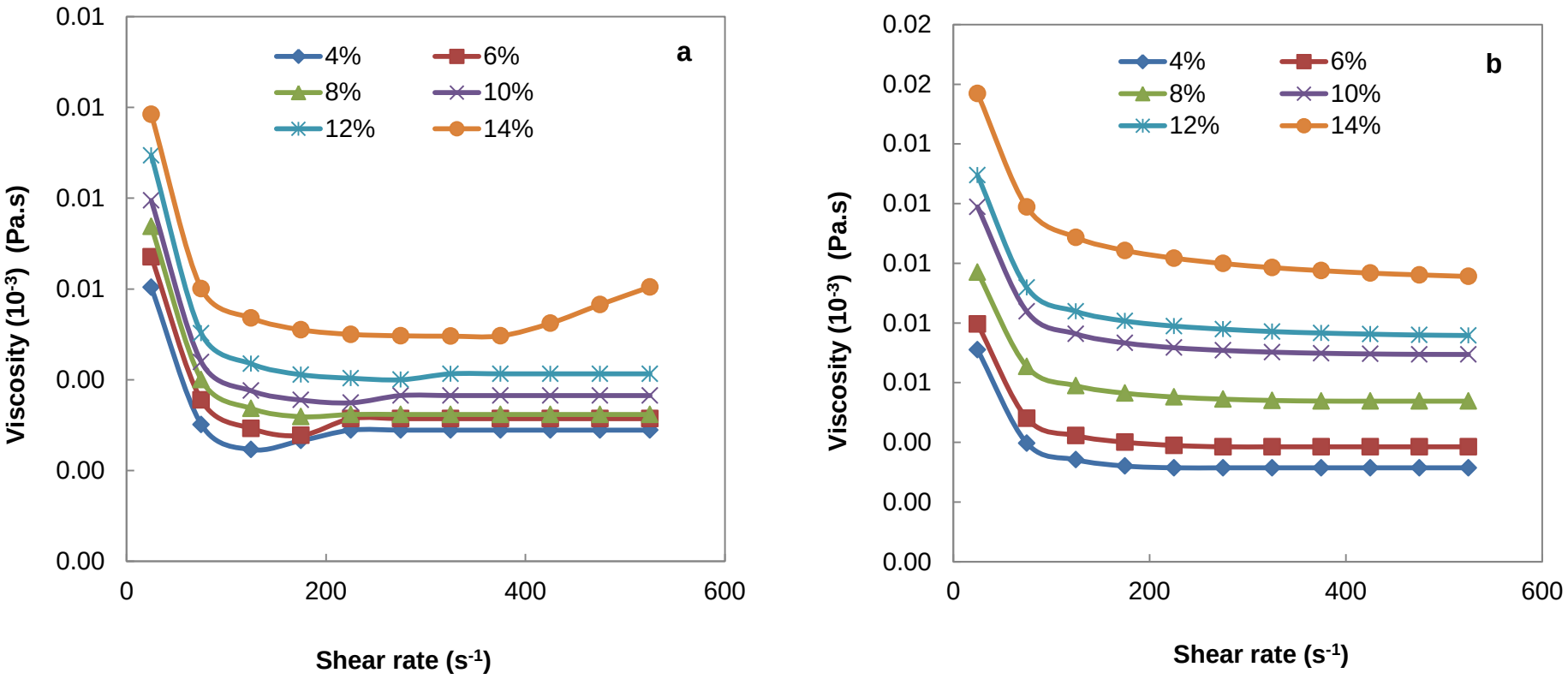

Figure 3.9 Viscosity of Bambara groundnut soluble dietary fibres (a) Black-eye (b) Brown-eye.

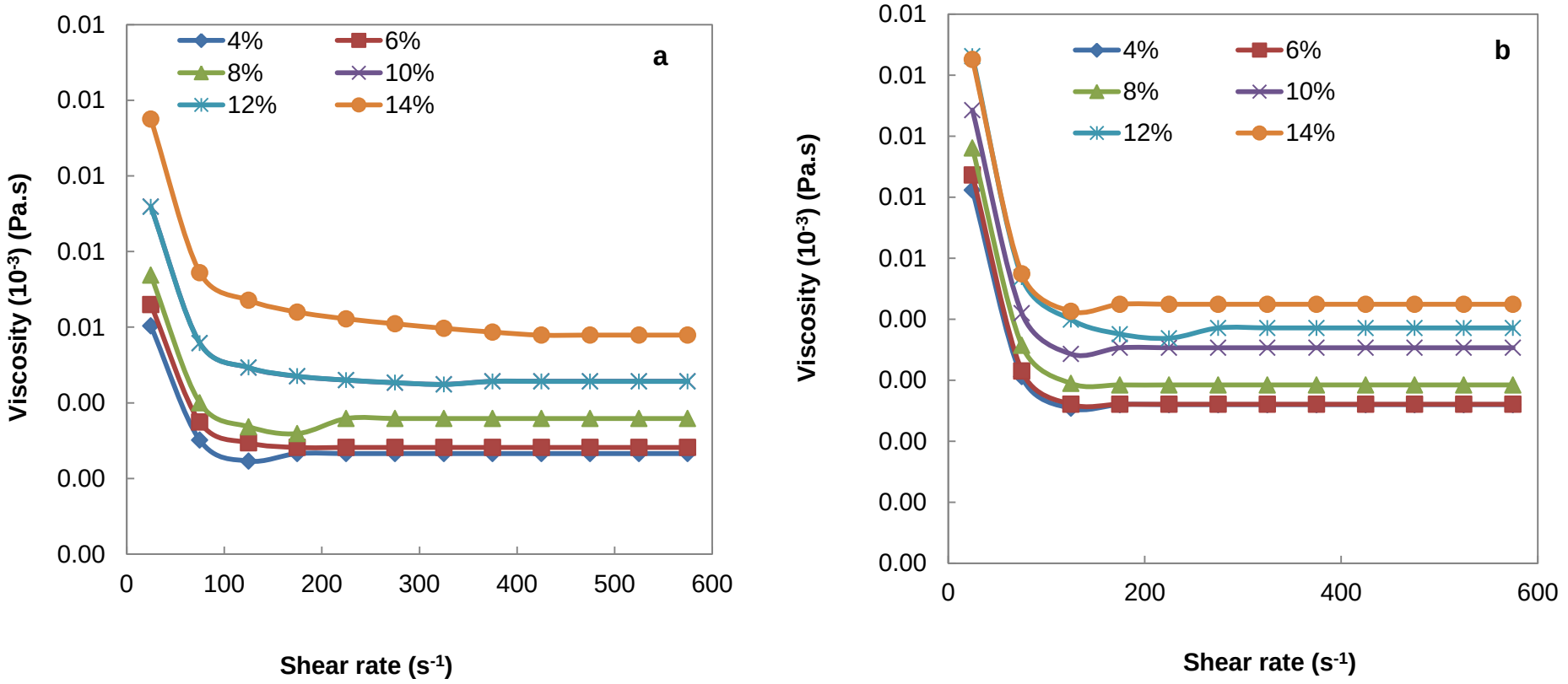

Figure 3.10 Viscosity of Bambara groundnut soluble dietary fibres (a) Brown (b) Red.

The rheograms (Figures 3.9 and 3.10) showed that all four SDFs displayed shear thinning behaviour evidenced by the gradual decrease in apparent viscosities with increasing shear rate. The shear thinning behaviour of BGN SDFs can be attributed to the gradual rearrangement of fibre strands during the shearing process making their flow easier (Soukoulis *et al*., 2009). The decrease in apparent viscosity observed in all SDFs was an indication of structural damage and the results of this study are confirmed by the time dependent experiments [Section 3.4.8 (1), page 84] where the hysteresis loop areas proved that BGN SDF structures degraded with increasing shear. In a related study, Adeyi (2014) reported a decrease in viscosity of BGN flour with increasing shear rate. The behaviour exhibited by BGN SDFs is widely encountered in food systems (Izidoro *et al.*, 2009) and is shown by most hydrocolloids (Mathur & Mathur, 2005). Guillon & Champ (2000) reported that the viscosity of fibres decreased with increasing shear rate with the exception of very dilute systems. Viscosities of polysaccharides are dependent on factors such as temperature, solvent and concentration (Guillon & Champ, 2000).

At a shear rate 200 s^{-1}, 4%, 6% and 8% concentrations of black-eye SDF (Figure 3.9a) as well as the 4% and 6% of red SDF (Figure 3.10b) showed similar behaviours. Black-eye SDF (14%) showed sudden thickening at shear rate of about 400 s^{-1}. This could suggest that molecular bonds that had been broken during shearing were attempting to interact and reform or form new bonds. This would make the system more reluctant to flow hence the observed thickening (Adeyi, 2014).

Ramirez-Santiago *et al.* (2010) evaluated the rheological properties of yoghurt enriched with *Pachyrhizus erosus* (yam) SDF. The researchers concluded that the SDF was responsible for the increase in viscosity and the more pronounced shear thinning properties that eventually helped to develop a better mouthfeel. BGN fibres exhibited the same rheological characteristics hence it can be deduced that they can also be incorporated in yoghurts and possibly other dairy products to increase viscosity and improve mouthfeel. Ahmed *et al.* (2012) evaluated the rheological properties of bread prepared from wheat flour-lupin fibre dough blends and observed a decrease in flow-ability with increase in concentration. The researchers attributed this to the viscosity properties of the added fibre. Hence, since BGN SDFs exhibited the same properties, they too could be included in dough to increase the rheological properties of bread and possibly other related baked products. BGN SDFs would be expected to increase the viscosities of systems by increasing the total solids (Soukoulis *et al*., 2009)

3. *Viscoelastic properties of BGN soluble dietary fibres*

Viscoelasticity describes the existence of the viscous and elastic properties in a material (Barbosa-Canovas *et al.,* 1995). The oscillatory behaviour of BGN SDFs at different frequencies is depicted in Figures 3.11 and 3.12. Higher positioned lines on the rheograms

indicate more stability at a certain frequency compared to lower positioned lines (Adeyi, 2014). Only loss modulus (G'') was observed for 4 - 12% concentrations for all the SDFs. The absence of storage modulus (G') indicated the absence of elastic properties within the SDFs at the mentioned concentrations. If BGN SDF concentrations less than 14% were used in food systems such as bakery products, yoghurts or emulsions, they would not be expected to impart any elastic properties but would contribute to the viscosity of the system.

At high frequencies, black-eye SDF showed little differences in loss modulus (G") at all concentrations (Figure 3.11a). This behaviour at high frequencies could be cost effective to the manufacturer as a less concentration can be used and still render the similar desirable behaviour when subjected to stresses such as mixing or blending. At high frequencies, 6% brown-eye SDF exhibited better viscosity and stability properties than 4% however at lower frequencies they exhibited similar behaviours. Therefore, at low frequencies, such as in storage in a refrigerator over prolonged periods of time, structural interactions and rearrangements may cause the dietary fibre structures to weaken and possibly dissociate. Brown-eye SDF (14%) would be expected to be stable for the longest periods. Brown SDF showed a higher stability associated with increase in concentration (Figure 3.12b). In this case, a higher concentration would be preferable in a food system that undergoes a lot of stress.

During transport, distribution, handling and processing, foods are exposed to various stresses which affect their structures (Gough, 2013). The rate of disruption is dependent on the strength of the system. The lowest concentration on each rheogram (Figures 3.11 and 3.12) would be most susceptible to disruption and the highest concentration would be expected to hold the structure and maintain its integrity for longer periods (Adeyi, 2014). The 4% concentration of black-eye (Figure 3.11a) and red (Figure 3.12b) SDFs could not be plotted as their data contained negative values.

4. *Viscoelastic properties of 14% solutions of BGN fibres*

The storage modulus (G') was only observed for 14% of SDFs from all varieties. The rheograms (Figures 3.13 and 3.14) show the storage modulus (G') indicating elastic properties and the loss modulus (G'') indicating viscosity properties of all four BGN SDF varieties. At low frequencies, if storage modulus (G') is positioned above loss modulus (G'') it is an indication that the structure is stable and if storage modulus (G') is positioned below loss modulus (G'') it is an indication that the structure is susceptible to dissociation (Adeyi, 2014).

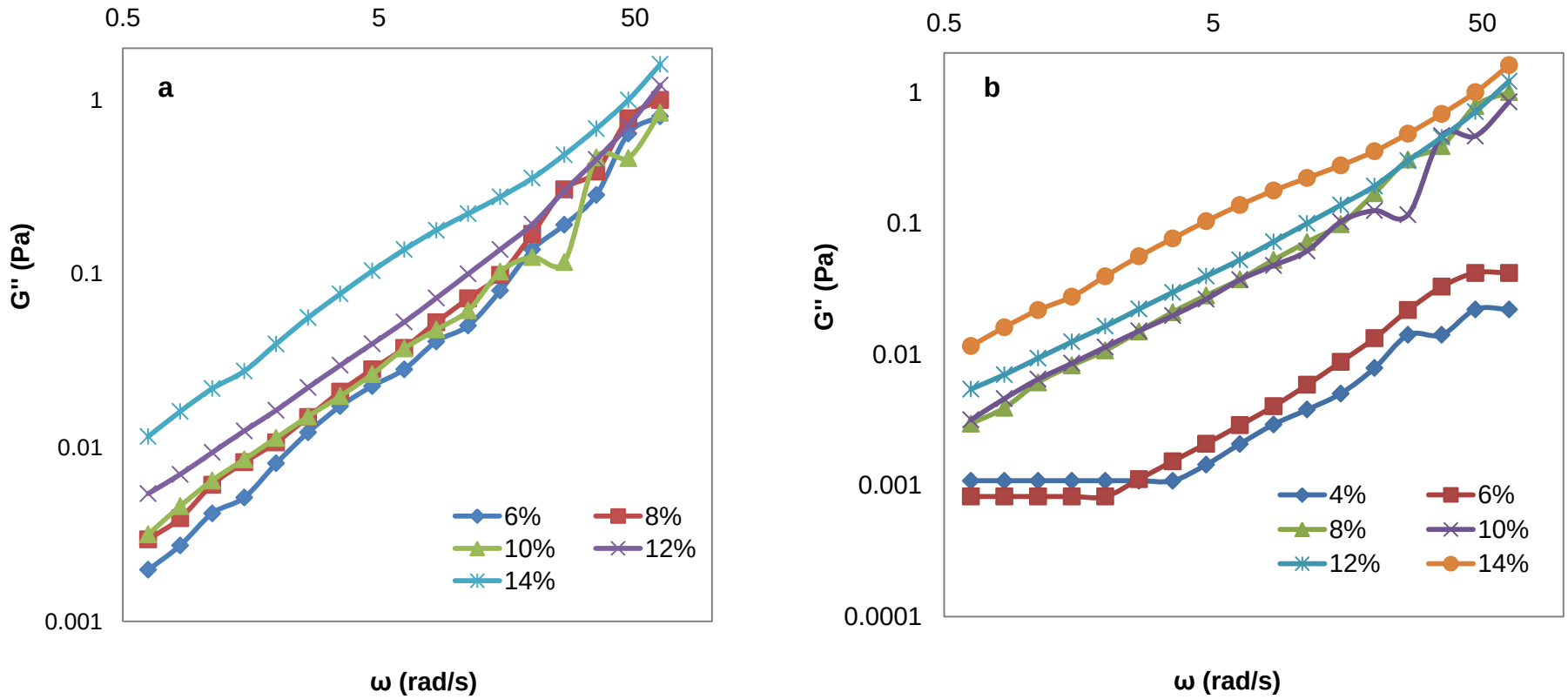

Figure 3.11 Viscoelastic properties of Bambara groundnut soluble dietary fibres (a) Black-eye (b) Brown-eye. G'': loss modulus.

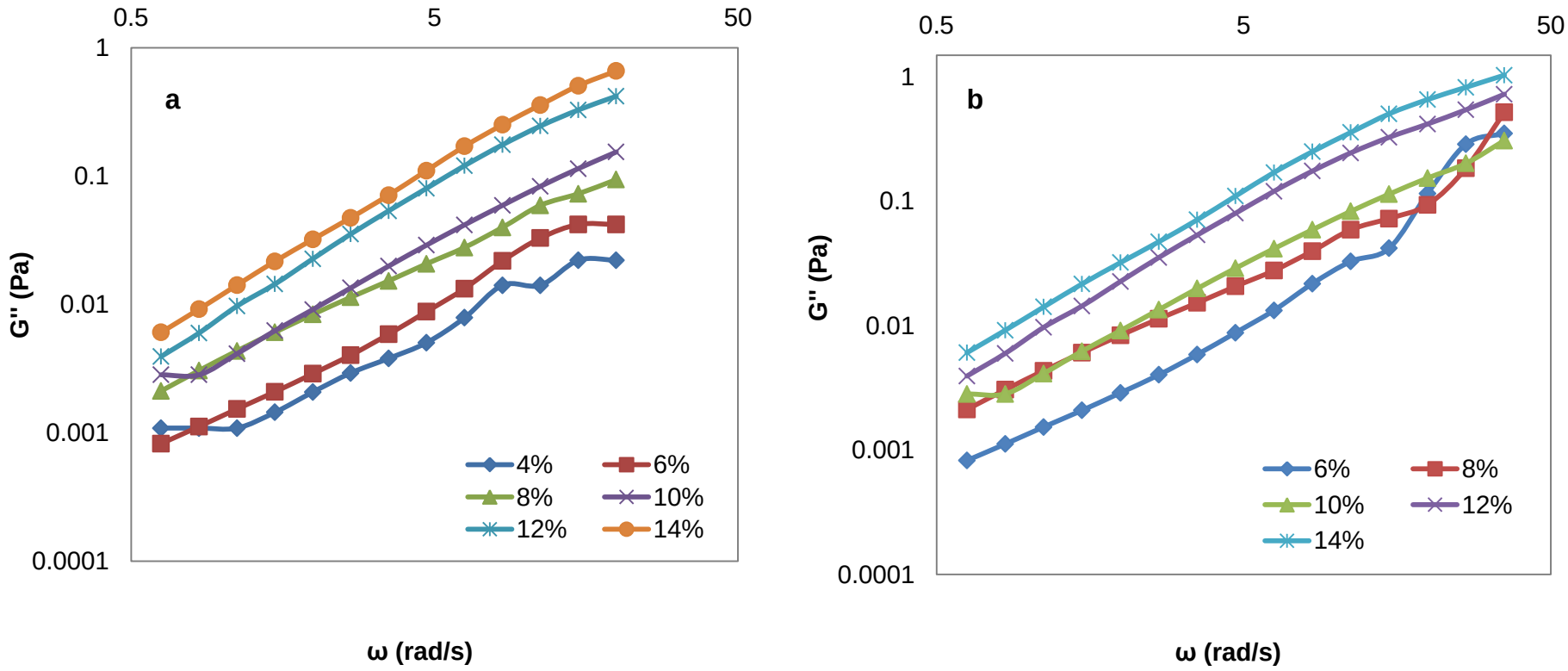

Figure 3.12 Viscoelastic properties of Bambara groundnut soluble dietary fibres (a) Brown (b) Red. G'': loss modulus.

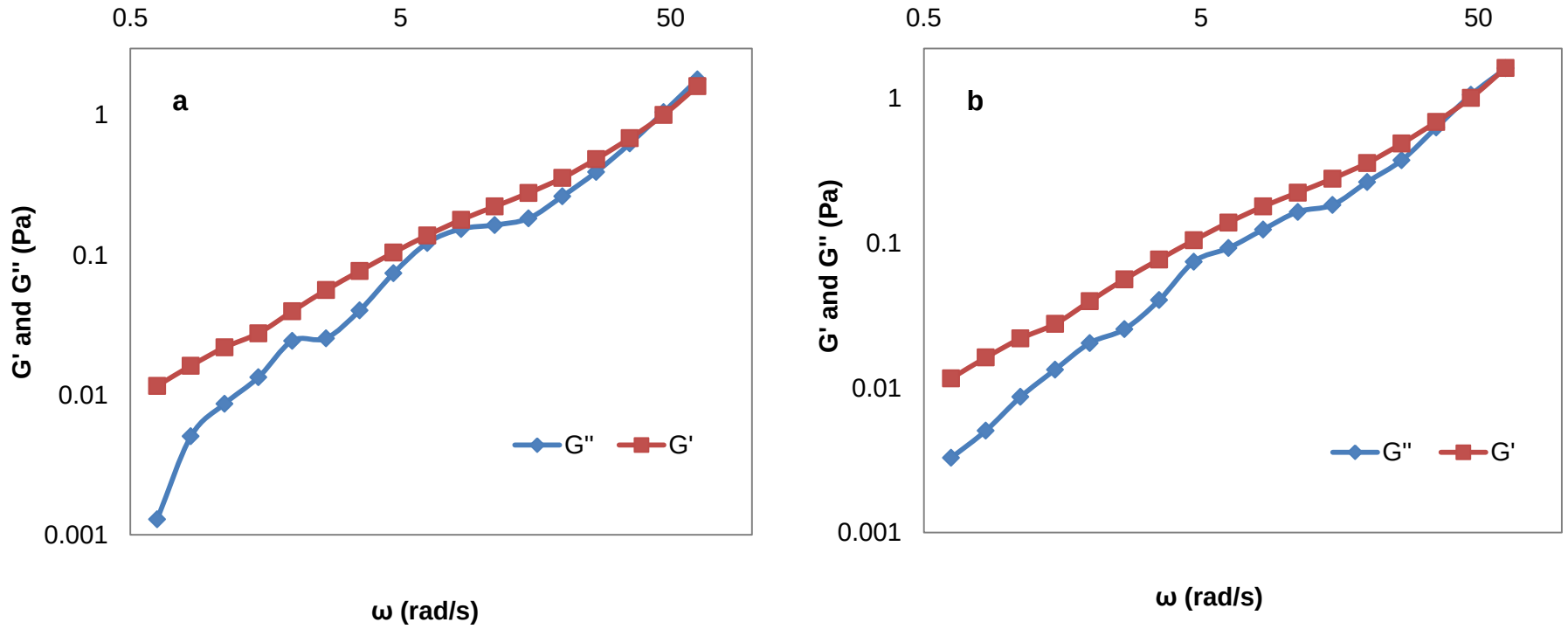

Figure 3.13 Viscoelastic properties of 14% Bambara groundnut soluble dietary fibres (a) Black-eye (b) Brown-eye. G': storage modulus. G'': loss modulus.

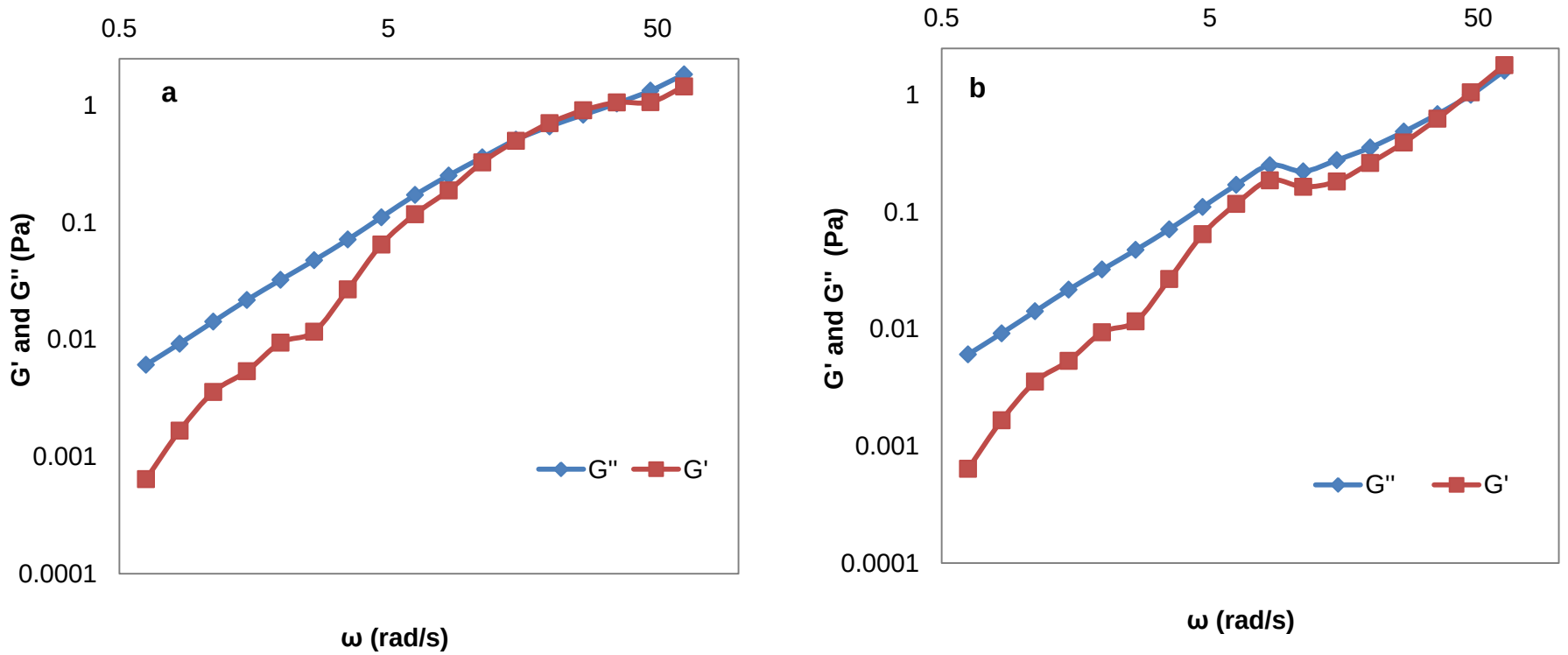

Figure 3.14 Viscoelastic properties of 14% Bambara groundnut soluble dietary fibres (a) Brown (b) Red. G': storage modulus. G'': loss modulus

Black-eye (Figure 3.13a) and brown-eye (Figure 3.13b) SDFs showed storage modulus (G') positioned above loss modulus (G'') while brown (Figure 3.14a) and red (Figure 3.14b) SDFs showed storage modulus (G') positioned below loss modulus (G''). This indicated that elastic properties of red and brown SDFs cannot be harnessed in food systems such as emulsions and yoghurts as they would exhibit a liquid character and form unstable structures (Mezger, 2006).

At higher frequencies (Figures 3.13a and 3.13b) loss modulus (G'') crosses over storage modulus (G'); at this point the system would be expected to start losing its stability (Adeyi, 2014). In this study, SDFs showed an increase in storage modulus (G') and loss modulus (G'') with increasing frequency. This was in fair agreement with Ahmed *et al.* (2012) but in contrary to Rasper (1993) and Derkach (2009) who stated that higher frequencies resulted in decrease in storage modulus (G') and loss modulus (G'') due to a conversion from viscoelastic solid to elastoviscous liquid.

With reference to literature, it can be argued that since black-eye and brown-eye SDFs possess viscoelastic properties characteristics of other previously studied fibres; they too can find use in food applications (Gallegos *et al.,* 2004; Piteira *et al.*, 2006; Lorenzo *et al.*, 2008; Nikovska *et al.*, 2010; Ahmed *et al*., 2012). The viscoelastic properties of BGN SDFs can only be obtained at higher concentrations (14%). This observation is in agreement with Gallegos *et al.* (2004) who reported that an increase in concentration of a thickening agent is associated with higher viscosity and viscoelastic function.

3.4.9 Heat stability of BGN fibres

The thermal behaviour of BGN fibres is given in Table 3.9. Two major peaks were observed for the BGN SDFs (Table 3.9). The onset of the first peak ranged from 236.59°C (black-eye SDF) to 269.43°C (brown SDF). The peaks of black-eye, brown-eye and red SDFs reached a maximum temperature of 275°C and that of brown SDF reached a temperature of 291.47°C. The temperature at the end of the peaks was in the range 239.52°C (brown SDF) to 299.34°C (black-eye SDF). All the changes in enthalpy (ΔH) of the SDFs were negative in the range -44.43 J/g (brown SDF) to -203.78 J/g (black-eye SDF). The changes in enthalpy (ΔH) of brown-eye and brown SDFs as well as of black-eye and red SDFs did not differ significantly ($p > 0.05$). Minor undulations were observed in the thermographs of SDFs at temperatures below 100°C, and could be attributed to loss of humidity which could have been absorbed by the hygroscopic SDFs (Bernabe *et al.*, 2013).

Assessment of thermal properties is of importance so as to establish a relationship between temperature and specific physical properties (Gill *et al.*, 2010). The data collected shows the behaviour of a particular material when exposed to high temperatures.

Table 3.9 Thermal properties of Bambara groundnut dietary fibres

	Peak 1				Peak 2			
Variety	**Onset (°C)**	**Peak (°C)**	**End (°C)**	**∆H (J/g)**	**Onset (°C)**	**Peak (°C)**	**End (°C)**	**∆H (J/g)**
IDF								
Black-eye					322.72	342.79	358.99	-161.00[a]
Brown-eye					312.96	341.31	373.95	-545.17[b]
Brown					305.50	340.17	366.29	-351.11[c]
Red					304.65	341.74	372.91	-526.65[d]
SDF								
Black-eye	236.59	275.68	299.34	-101.89 ± 2.02[a]	342.78	361.51	371.08	-244.65[a]
Brown-eye	251.64	275.52	296.63	-86.24 ± 0.16[b]	321.60	360.25	403.69	-304.20[b]
Brown	269.43	291.47	239.52	-44.43 ± 0.91[c]	320.60	358.36	388.22	-372.49[c]
Red	254.02	275.65	298.89	-97.07 ± 1.37[d]	342.38	361.51	371.97	-275.90[d]

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [p > 0.05] different;
IDF: Insoluble dietary fibre; SDF: Soluble dietary fibre; ∆H: Enthalpy change of reaction.

Zhang & Wang (2013) observed degradation peaks between 242.83°C to 280.91°C for Canna edulis Ker SDFs. These degradation temperatures are comparable to the first degradation of the BGN SDFs. As such, BGN SDFs have similar thermal stabilities as Canna edulis Ker SDFs. The second degradation peak of SDFs ranged from 305.50°C (brown SDF) to 322.72°C (black-eye SDF). The peaks reached a maximum range of 358.36°C (brown SDF) to 361.51 (black-eye and red SDFs). The end of the peaks was in the range 358.99°C (black-eye SDF) to 373.95°C (brown-eye SDF). All the changes in enthalpy (ΔH) were negative and differed significantly ($p < 0.05$) in the range -244.65 J/g (black-eye SDF) and -372.49 J/g (brown SDF).

Only one major peak was observed in the degradation of IDFs (Table 3.9). The onset of the peaks ranged from 320.60°C (brown IDF) to 342.78°C (black-eye IDF). The peaks reached a maximum temperature of 304.65°C (red IDF) to 342.79°C (black-eye IDF) and the end of the peaks was in the range 371.08°C (black-eye IDF) to 403.69°C (brown-eye IDF). All the changes in enthalpy (ΔH) were negative and differed significantly ($p < 0.05$) in the range -161.00 J/g (black-eye) to -545.17 J/g (brown-eye) for IDFs.

The negative enthalpy changes of reaction (ΔH) observed for both BGN SDFs and IDFs indicated that the reactions were exothermic, meaning the fibres released energy as they combusted. This exothermic behaviour suggested that the fibres were charred instead of volatilised when they were degrading (Yang *et al.*, 2007). Degradation of BGN dietary fibres at temperatures above 300°C indicated high thermal stability and suggested the presence of strong intra- and intermolecular bonds between BGN fibre strands. These bonds could include H-H bonds, C-H bonds common in cellulose, hemicellulose and lignin as well as hydroxyl H-bonds that breakdown at temperatures above 300°C (Yang *et al.*, 2007; Ramanen *et al.,* 2012; Bernabe *et al.*, 2013). These saccharides are present in BGN dietary fibres according to the sugar composition shown in Table 3.6. In this study, BGN SDFs and IDFs started degrading at 236.59°C and 304.65°C, respectively. This indicated that these fibres are stable enough to withstand some thermal processing such as baking. BGN fibres could be used as ingredients in bakery products as the baking temperatures vary between 176°C and 250°C (Therdthai *et al*., 2002; Mondal & Datta, 2008). At higher temperatures, BGN IDFs would be preferred as they have higher thermal stabilities than SDFs.

3.4.10 Microstructure of BGN dietary fibres

Scanning electron images of BGN soluble and insoluble dietary fibres analysed at 500X magnification are shown in Figure 3.15. All dietary fibres consisted of particles of different shapes and sizes. The volume surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter ($d_{4,3}$) of each BGN SDF is given in Table 3.10. The volume surface mean diameter gives information regarding area where most particles fall whilst the equivalent volume-mean diameter measures changes in particle sizes (Adeyi, 2014).

The volume surface mean diameter ($d_{3,2}$) of BGN SDFs analysed at 500X magnification differed ($p < 0.05$) significantly and ranged from 73.05 µm (brown SDF) to 116.24 µm (black-eye SDF) and the equivalent volume-mean diameter ($d_{4,3}$) differed ($p < 0.05$) significantly and was between 74.74 µm (brown SDF) and 124.30 µm (black-eye SDF). Among the SDFs, black-eye SDF showed the largest particle size and brown SDF showed the smallest.

Among the IDFs, volume surface mean diameter ($d_{3,2}$) ranged from 78.38 µm (black-eye IDF) to 120.64 µm (brown IDF). Brown-eye and brown IDFs did not differ ($p > 0.05$) significantly in the volume surface mean diameter of their particles and were both significantly ($p < 0.05$) higher than red and black-eye IDFs in terms of volume surface mean diameter. The equivalent volume-mean diameter ($d_{4,3}$) of BGN IDFs differed ($p < 0.05$) significantly and ranged from 79.47 µm (black-eye IDF) to 125.42 µm (brown IDF). The results obtained in this study were in fair agreement with the research of Diedericks (2014) who analysed the structures of four varieties of BGN IDFs and reported brown IDF to be characterised by the largest particles and black-eye IDF by the smallest particles. Since all the fibres originated from the same source (BGN), the differences in particle sizes and particle size distribution could be attributed to varietal differences.

Equivalent volume-mean diameter ($d_{4,3}$) of cellulose, locust bean gum and guar gum (89.1 – 164.4 µm) reported by Rosell *et al.* (2009) were fairly comparable to those of the BGN fibres. Collar & Angioloni (2010) reported the volume surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter ($d_{4,3}$) of pea dietary fibres between 144 – 265.7 µm and 272.5 – 471.1 µm respectively. Pea dietary fibres have been shown to possess similar physicochemical properties to BGN dietary fibres (Dalgetty & Baik, 2003; Huang *et al*., 2009; Elleuch *et al*., 2011). The smaller particle sizes and particle size distribution makes BGN fibres more superior as they have larger surface areas hence increasing their functional properties both in food systems and in the gastrointestinal canal (Rosell *et al.*, 2009; Daou & Zhang, 2012).

The determination of particle sizes and particle size distribution of fibres is of importance as these factors largely affect the efficiency of processing, flow behaviour, texture, viscosities, physiological roles such as transit time and fermentation and may be indicators of quality and performance (Rosell *et al.*, 2009; Collar & Angioloni, 2010; Wang *et al.,* 2013). The range of particle sizes and particle size distribution is largely dependent on the type of cell walls present in the particular plant as well as degree of processing (Dhingra *et al.*, 2012).

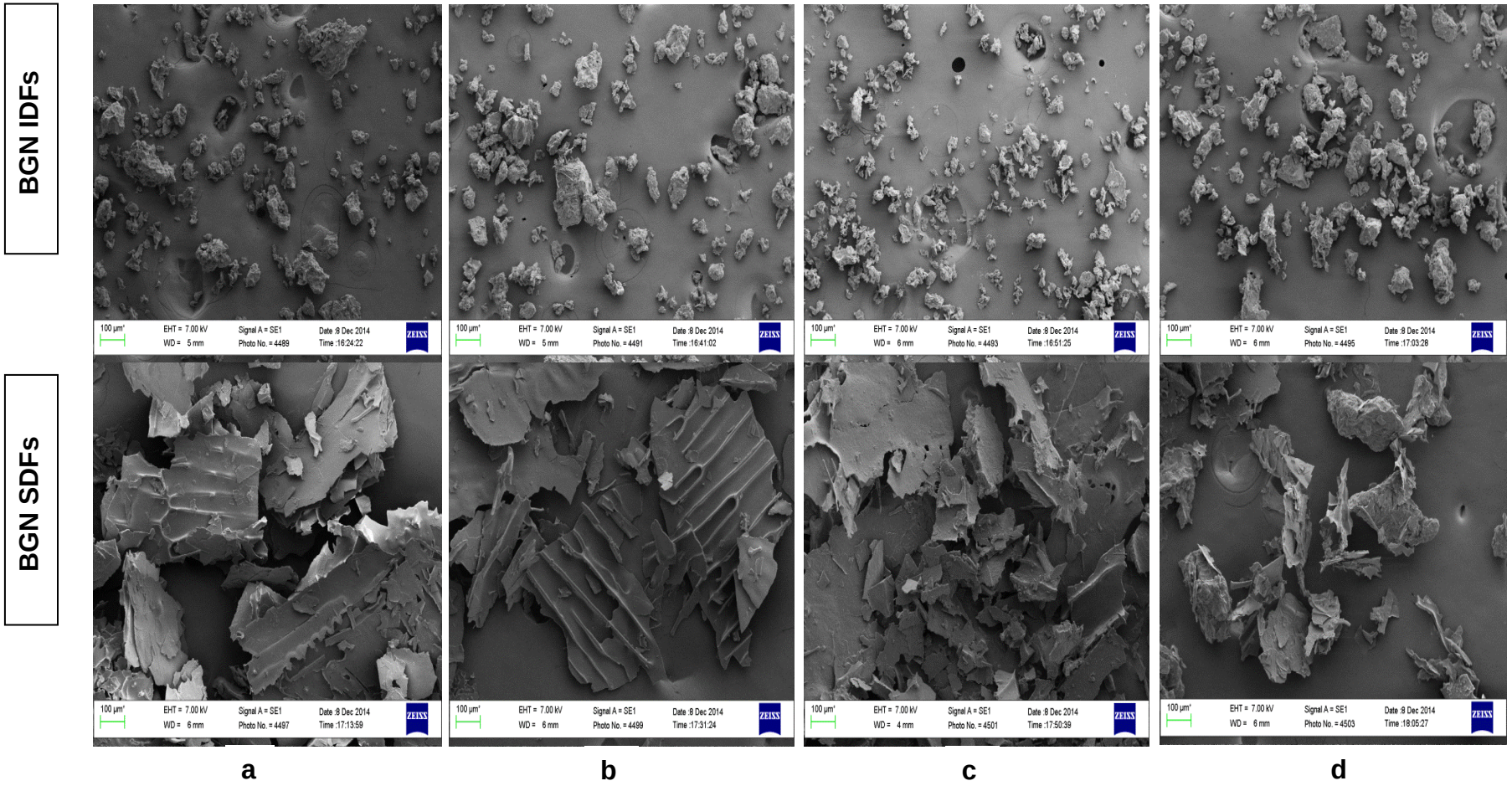

Figure 3.15 Scanning electron micrographs of soluble and insoluble dietary fibres (SDFs and IDFs, respectively) isolated from four varieties of Bambara groundnut (BGN) (a) Black-eye (b) Brown-eye (c) Brown (d) Red.

Table 3.10 Mean diameters of Bambara groundnut fibres analysed by SEM at 500X magnification

Variety	$d_{3,2}$ (µm)	$d_{4,3}$ (µm)
SDF		
Black-eye	116.24[a]	124.30[a]
Brown-eye	105.33[b]	111.19[b]
Brown	73.05[c]	74.74[c]
Red	116.19[d]	122.35[d]
IDF		
Black-eye	78.38[a]	79.47[a]
Brown-eye	120.69[b]	125.42[b]
Brown	120.64[b]	125.42[c]
Red	108.63[c]	111.61[d]

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [p > 0.05] different; IDF: insoluble dietary fibre; SDF: soluble dietary fibre. SEM: scanning electron microscope. $d_{3,2}$: volume surface mean diameter $d_{4,3}$: equivalent volume-mean diameter

3.5 Comparison of the wet milling with enzymatic-gravimetric methods

3.5.1 Extraction methods

The methodology of the wet milling method is discussed in Section 3.2.3 (page 54). The enzymatic-gravimetric method of Diedericks (2014) differs from the wet milling method in that it is an enzyme based extraction process while wet milling is a water based extraction method. Furthermore, a smaller sample of BGN flour (18 g) is used in the enzymatic-gravimetric method and hence it could be assumed that fibres of a higher purity are obtained.

3.5.2 Cost of fibre extraction using the enzymatic-gravimetric and the wet milling methods

The cost of chemicals, equipment and enzymes used in the application of each method is given in Table 3.11. The enzymatic-gravimetric method cost approximately ZAR26388.57/kg and the wet milling method cost approximately ZAR9136.02/kg of total dietary fibre (SDF and IDF). To obtain this amount of total dietary fibre, 56 extractions needed to be carried out in the enzymatic-gravimetric method whilst 5 extractions were carried out in the wet milling method.

The cost of a method directly imparts on the cost of the product, as such BGN dietary fibre obtained from the enzymatic-gravimetric method would cost more than that from wet milling method. The enzymatic-gravimetric method has a starting weight of 18 g of flour per run whilst the wet milling method has a starting weight of 200 g of flour per run. This would be a disadvantage in industry where very large quantities of dietary fibre would be required. Ways to upscale the starting extraction mass should be investigated so as to increase the advantage of commercially extracting BGN fibres.

Table 3.11 Cost of the enzymatic-gravimetric and wet milling methods

	Enzymatic-gravimetric method (ZAR/kg)	**Wet milling method (ZAR/kg)**
Cost of chemicals	8870.35	314.48
Cost of equipment*	7945.54	7945.54
Cost of enzymes	9572.68	876.00
Total	**26388.57**	**9130.48**

*Equipment refers to any machinery or instrument specifically purchased for use in the application of the method. ZAR: South African Rand.

Anon. (2006) reported the price of dietary fibre traded as AIM Herbal Fibreland™ as ZAR0.62/g. Nutrilite™ fibre powder costs ZAR2.16/g; this fibre is composed of a blend of three soluble fibres namely dextrin, inulin and guar gum (Anon., 2014). Dietary fibre from BGN would need to compete with such fibres in the market. Hence, a cheaper method of extraction would play a role in the reduction of its cost thereby giving it a competitive advantage. In order to calculate an accurate estimated pricing and profit of BGN fibre in the market, variable and fixed costs, contribution margin as well as the number of units and revenue to break even need to be taken into consideration.

3.5.3 Physicochemical properties of BGN fibres from the wet milling and enzymatic-gravimetric methods

Table 3.12 shows the comparison of the physicochemical properties of BGN dietary fibres from the wet milling and enzymatic-gravimetric methods. The physicochemical properties of BGN soluble dietary fibres (SDFs) and insoluble dietary fibres (IDFs) extracted from both methods were compared using least significant difference (LSD) on IBM Statistical Package for the Social Science (IBM SPSS, version 22, 2013). The following comparisons were made:

1. Swelling capacities (6.37 – 7.72 g/mL) of BGN IDFs reported for fibres from the enzymatic-gravimetric method, were significantly ($p \leq 0.05$) higher than those from the wet milling method (5.5 - 6.5 g/mL). The high swelling capacities of BGN dietary fibres was indicative that these fibres could applied as bulking agents, stabilisers, thickeners and anti-caking agents in food systems as well as play an important physiological role by reducing constipation (Rosell *et al.*, 2009).
2. Water holding capacities (1.63 - 2.01 g water/ g sample) of BGN IDFs from the enzymatic-gravimetric method were significantly ($p < 0.05$) lower than those from the wet milling method (2.41 - 2.84 water/ g sample). BGN IDFs can thus find use in bakery and dairy products, maintaining freshness for longer and reducing syneresis, respectively. Physiologically, BGN IDFS would play an important role in increasing laxation and improves peristalsis (Esposito *et al.*, 2005; Elleuch *et al*., 2011). BGN IDFs from wet milling would be expected to have a better performance than those from enzymatic-gravimetric method both in food systems and in the human body.
3. Bulk densities for IDFs (0.82 – 0.86 g/mL) and SDFs (0.81 – 0.93 g/mL) from the enzymatic-gravimetric method were significantly ($p < 0.05$) higher than those from the wet milling method (IDFs: 0.57 - 0.67 g/mL and SDFs: 0.46 - 0.57 g/mL). The lower densities of BGN SDFs from wet milling method imply that they will pack closer together hence requiring less packaging material, consequently reducing packaging costs as well as storage and distribution space (Diedericks, 2014).

Table 3.12 Physicochemical properties of Bambara groundnut dietary fibres from the wet milling and enzymatic-gravimetric methods

Physicochemical properties	**Wet milling**	**Enzymatic-gravimetric method**
Water holding capacity (g water/g fibre)	2.84 ± 0.08[a]	2.01 ± 0.04[b]
Oil binding capacity (g oil/g dry)	1.40 ± 0.07[a]	1.49 ± 0.03[a]
Swelling capacity (mg/g)	6.17 ± 0.29[a]	6.83 ± 0.29[b]
Bulk density (g/ml)	0.57 ± 0.00[a]	1.22 ± 0.29[b]
Lightness	36.61 ± 0.39[a]	65.81 ± 0.02[b]
Redness/Greenness	9.89 ± 0.09[a]	6.68 ± 0.01[b]
Yellowness/Blueness	17.61 ± 0.32[a]	19.05 ± 0.00[b]

Values are mean ± standard deviation. Means within a row followed by the same subscript are not significantly [p > 0.05] different.

4. Oil binding capacities (OBCs) of BGN IDFs (1.38 – 1.52 g oil/ g sample) and SDFs (4.55 g oil/ g sample) from the enzymatic-gravimetric method did not differ significantly ($p > 0.05$) from those from the wet milling method (IDFs: 1.40 - 1.52 g oil/g sample and SDFs: 2.78 - 4.03 g oil/ g sample). The OBC of BGN fibres would be of importance in stabilising emulsions and as fat binders in food systems (Slavin, 2013). Physiologically, BGN fibres could play a role in bile acid absorption resulting in cholesterol reduction (Tosh & Yada, 2010).
5. BGN dietary fibres from the enzymatic-gravimetric method (65.81) were significantly ($p < 0.05$) lighter in colour than those from the wet milling method (36.61). The wet milling method is a water based extraction method while the enzymatic-gravimetric method subjects the sample to a succession of enzymatic digestions and chemical treatments (Diedericks, 2014). These chemicals bleach the fibres resulting in a lighter colour. The disadvantage of bleaching is that important colour pigments associated with phenolic compounds are lost hence reducing the antioxidant capacity of the fibres. This was evidenced by a significantly ($p < 0.05$) lower redness/greenness (6.68) associated with BGN fibres from the enzymatic-gravimetric method.
6. Xylose, arabinose/galactose and mannose were the main sugars in IDFs while mannose and xylose were the main sugars in SDFs reported in both studies. Rhamnose was absent in dietary fibres from the enzymatic-gravimetric method but was present in IDFs from the wet milling method therefore suggesting the presence of rhamnogalactans. The presence of rhamnose and galactose is indicative of the presence of rhamnogalacturonans, which are important in binding heavy metals in the human body as well as lower blood cholesterol (Huang *et al.*, 2009; Sivam *et al.*, 2010; Khotimchenko *et al.*, 2012).
7. Lower amounts of tannins, below 2.2 mg/g in IDFs were reported in both studies which was desirable because tannins bind to proteins thus interfering with their digestion. In addition, tannins by forming complex with other nutrients that cannot be processed by the body (Saura-Calixto & Bravo, 2001).
8. BGN SDFs from wet milling were significantly ($p < 0.05$) higher in uronic acid content than those from enzymatic-gravimetric method. Uronic acid play a major role in the body's detoxification by complexing with compounds such as drugs hence facilitating their excretion from the body (Vazquez *et al.*, 2013). Uronic acids are also building units of mucopolysaccharides which are major components of body tissues and fluids (Chhabra, 2012). Therefore, BGN SDFs from wet milling will effectively play these roles.

The comparisons of the physicochemical characteristics of the dietary fibres extracted using the two methods showed that the wet milling method produces a higher yield of fibres

with a higher quality than the enzymatic-gravimetric method. The hydration properties (water holding capacities and swelling capacities), oil binding capacities, antioxidant properties, densities, total sugar and uronic acid content of BGN dietary fibres revealed their potential for various applications in food systems.

3.6 Conclusions

The wet milling method was successfully applied in the extraction of soluble and insoluble dietary fibre fractions of BGN yielding an appreciable amount of both fractions. The wet milling method is not only more cost-effective than the enzymatic-gravimetric method but also yields more BGN DFs. Black-eye and brown-eye fibres have superior physicochemical properties compared to the brown and red fibres as evidenced by their higher Swelling capacities,water holding capacities, oil binding capacities, antioxidant and total sugar content as well as their superior rheological and thermal stabilities. BGN fibres could also be of physiological importance due to their hydration, bulking and uronic acid properties. Black-eye and brown-eye insoluble dietary fibres were lighter in colour, yellower, redder, more saturated and had higher hues compared to the brown and red fibres. This could be of importance in instances where a noticeable colour change to the product is not desired. The increase in viscosity of BGN fibres is directly related to the increase in concentration. Furthermore, viscoelastic properties were only associated with 14% fibre concentration. It can be concluded that higher concentrations of BGN fibre would render more stable and viscous systems. The physicochemical properties of BGN fibres make them valuable to the food industry as potential fortifiers, thickening agents, gelling agents as well as cryoprotectants in frozen dairy products. BGN fibres can be considered suitable alternatives for commercial fibres such as pea, chick pea and lentil fibres as they have been shown to possess similar qualities to these fibres. BGN fibres would be suitable for high temperature applications such as baking.

References

Adeyi, O. (2014). Effect of Bambara Groundnut flour on the stability and rheological properties of oil-in-water emulsion. PhD: Chemical Engineering Thesis, Cape Peninsula University of Technology.

Ahmed, A.R., Mohammed, I. & Senge, B. (2012). Oscillation Measurements and Creep Test of Bread Prepared from wheat-Lupin flours and Wheat Flour-Lupin Fibre Dough's Blends. *Annual Transactions of the Nordic Rheology Society,* (**20**), 145 - 152.

Anonymous. (2006). Herbal Fibreblend dietary fibre. http://totalhealth.co.za/dietary-fibre/dietary-fibre.htm. 10 October 2014.

Anonymous. (2010). Processing methods for dry peas, lentils & chickpeas. In: *Processing Information & Technical Manual*. Moscow: USA Dry Pea & Lentil Council.

Anonymous. (2011). Department of Agriculture, Forestry and Fisheries. Directorate Production/ Plant Production. [Internet document] URL: http://www.nda.agric.za/docs/Brochures/ProdguideBambara.pdf. Accessed (25/01/2014).

Anonymous. (2014). Nutrilife™ Fibre Powder. https://www.amway.co.za/ Product/ Detail/102736?c=EN-ZA. 08 October 2014.

Barbosa-Canovas, G.V., Kokini, Z.L., Ma, L. & Ibarz, A. (1995). The Rheology of Semi Liquid Foods. *Advances in Food and Nutrition Research*, **39**, 1 - 69.

Baryeh, E. A. (2001). Physical properties of Bambara groundnuts. *Journal of Food Engineering,* **47**, 321 - 326.

Bernabe, G.A., Kobelnik, M., Almeida, S., Ribeiro, C.A. & Crespi, M.S. (2013). Thermal behavior of lignin and cellulose from waste composting process. *Journal of Thermal Analysis and Calorimetry*, **111**, 589 - 595.

Betancur-Ancona, D., Perza-Mercado, G., Moguel-Ordonez, Y. & Fuertes-Blanco, S. (2004). Physicochemical characterisation of lima beans (Phaselous lunatus) and jack bean (Canavalia ensiformis) fibrous residues. *Food Chemistry,* **84**, 287 - 295.

Bojnanska, T., Tokar, M. & Francakova, H. (2014). Changes of the Dough Rheological Properties Influences by Addition of Potato Fibre. *Potravinarstvo Scientific Journal for Food Industry,* **8**(1), 161 – 166.

Chaplin, M.F. (2003). Fibre and water binding. *The Proceedings of the Nutritional Society,* **62**(1), 223 – 227.

Chau, C.F. & Huang, Y.L. (2004). Characterization of passion fruit seed fibres - a potential fibre source. *Food Chemistry*, **85**, 189 - 194.

Chau, C.F., Huang, Y.L. & Chang, F.Y. (2005). Effects of fibre derived from passion fruit seed on the activities of ileum mucosal enzymes and colonic bacterial enzymes in hamsters. *Journal of the Science of Food and Agriculture,* **85**, 2119 - 2124.

Chhabra, N. (2012). Uronic Acid pathway. In: *Biochemistry for Medics*. Medical College, Mauritius.

Cheynier, V. (2005). Polyphenols in foods are more complex than often thought. *American Society for Clinical Nutrition,* **81**(1), 2235 – 2295.

Collar, C. & Angioloni, A. (2010). An approach to structure-function relationships of polymeric dietary fibres in foods: Significance in break making applications. In: *Dietary fibre: New frontiers for food and health.* (Edited by J.W. van der Kamp., J. Jones., B. McCleary. & D. Topping). Pp. 101. Wageningen: Wageningen Academic Publishers.

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

Dalgetty, D.D. & Baik, B. (2003). Isolation and characterisation of cotyledon fibres from Peas, Lentils, and Chickpeas. *American Association of Cereal Chemists*, **80**(3), 310 - 315.

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

Delgado-Vargas, F., Jimenez, A.R. & Paredes-Lopez, O. (2000). Natural pigments: Carotenoids, anthocyanins and betalains – characteristics, biosynthesis, processing and stability. *Critical Reviews in Food Science and Nutrition*, **40**(3), 173 – 289.

Derkach, S.R. (2009). Rheology of emulsions. *Advances in Colloid and Interface Science,* **151**, 1 - 23.

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.

Diedericks, C.F. (2014). Functional properties of Bambara groundnut (*Vigna Subterranea* (L) Verdc.) non-starch polysaccharides in model and food systems. Master of Technology: Food Technology Thesis, Cape Peninsula University of Technology.

Dragovic-Uzelac, V., Savic, Z., Brala, A., Levaj, B., Kovacevic, D.B. & Bisko, A. (2010). Evaluation of phenolic content and antioxidant capacity of blueberry cultivars (*Vaccinium corymbosum* L.) grown in the Northwest Croatia. *Food Technology and Biotechnology,* **48**(2), 214 – 221.

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

Esposito, F., Arlotti, G., Bonifati, A.M. Napolitano, A., Vitale, D. & Fogliano, V. (2005). Antioxidant activity and dietary fibre in durum wheat bran by-products. *Food Research International,* **38**, 1167 - 1173.

Fasoyiro, S.B., Ajibade, S.R., Omole, A.J., Adeniyan, O.N. & Farinde, E.O. (2006). Proximate, mineral and anti-nutritional factors of some underutilized grain legumes in South- Western Nigeria. *Nutrition and Food Science,* **38**, 18 - 23.

Fasoyiro, S., Yudi, W. & Taiwo, K. (2012). Processing and Utilization of Legumes in the Tropics. In: *Trends in Vital Food and Control Engineering*. (Ed. A.A. Eissa). Croatia: InTech.

Foodstuffs, Cosmetics and Disinfectants Act. (1972). Regulations Governing The Labelling And Advertising Of Foodstuffs, Regulation No. R146. In: Foodstuffs, Cosmetics and Disinfectants Act and Regulations, 54/1972. Updated 1 March 2010. Cape Town, Johannesburg: LexNexis Butterworths.

Gallegos, C., Franco, J.M. & Partal, P. (2004). Rheology of food dispersions. *The British Society of Rheology*, 19 - 65.

Gill, P., Moghadam, T.T. & Ranjbar, B. (2010). Differential scanning calorimetry techniques: Applications in biology and nanoscience. *Journal of Biomolecular Techniques*, **21**(4), 167 - 193.

Golenser, J., Frankenburg, S., Ehrenfreund, T. & Domb, A.J. (1999). Efficacious Treatment of Experimental Leishmaniasis with Amphotericin B-Arabinogalactan Water-Soluble Derivatives. *Antimicrobial Agents Chemotherapy,* **43**(9), 2209 - 2214.

Gomez-Romero. J., Garcia-Pena, I., Ramirez-Munos. J. & Torres, L.G. (2014). Rheological Characterisation of a Mixed Fruit/Vegetable Puree Feedstock for Hydrogen Production by Dark Fermentation. *Advances in Chemical Engineering and Science,* **4**(1), 81 – 88.

Gordon, D. T. & Okuma, K. (2002). Determination of Total Dietary Fibre in Selected Foods Containing Resistant Maltodextrin by Enzymatic-Gravimetric Method and Liquid Chromatography: Collaborative Study. *Journal of the Association of Analytical Chemists International*, **85**, 435 - 444.

Guillon, F. & Champ, M. (2002). Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International*, **33**, 233 - 245.

Hawkes C. (2006). Uneven dietary development: linking the policies of globalization with the nutrition transition, obesity, and diet-related chronic diseases. *Globalization and Health*, **2**, 4.

Hu, Q.P. & Xu, J.G. (2011). Profiles of carotenoids, anthocyanins, phenolics and antioxidant activity of selected color waxy corn grains during maturation. *Journal of Agriculture and Food Chemistry,* **59***,* 2026 – 2033.

Huang, S.C., Lia, T.S., Cheng, T.C., Chan, H.Y., Hwang, S.M. & Hwang, D.F. (2009). *In vitro* interactions on glucose by different fibre materials prepared from mung bean hulls, rice bran and lemon pomace. *Journal of Food and Drug Analysis,* **17**, 307 - 314.

IBM Corp. (2013). IBM Statistical Package for the Social Science (SPSS), Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Izidoro, D.R., Scheer, A. & Sierakowski, M. (2009). Rheological properties of emulsions stabilized by green Banana (Musa cavendishii) pulp fitted by power law model. *Brazilian Achives of Biology and Technology,* **52**, 1516 - 8913.

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). p 187. Croatia: InTech.

Jideani, V.A. & Mpotokwane, S.M. (2009). Modelling of water absorption of Botswana Bambara varieties using Peleg's equation. *Journal of Food Engineering*, **92**(2), 182-188.

Khan, A.R., Alam, S., Ali, S., Bibi, S. Khalil, I.A. (2007). Dietary fibre profile of food legumes. *Sarhad Journal of Agriculture*, **23**(3), 763 - 766.

Khalil, M. & Jan, B.M. (2011). Herschel-Bulkley rheological parameters of a novel environmentally friendly lightweight biopolymer drilling fluid from xanthan gum and starch. *Journal of Applied Polymer Science*, **124**(1), 595 - 606.

Khen, L.W. (2002). Color spaces and color-difference equations. Computer Science: National University of Singapore.

Khotimchenko, Y., Khozhaenko, E., Kovalev, V. & Khotimchenko, M. (2012). Cerium binding activities of pectins isolated from the seagrasses *Zostera marina* and *Phyllospadix iwatensis. Marine Drugs,* **10**(4), 834 – 848.

Kohajdova, Z., Karovicova, J. & Magala, M. (2013). Effect of lentil and bean flours on rheological and baking properties of wheat dough. *Chemical Papers*, **67**(4) 398 - 407.

Koocheki, A., & Razavi, S. M. A. (2009). Effect of concentration and temperature on flow properties of *Alyssum homolocarpum* seed gum solutions: assessment of time dependency and thixotropy. *Food Biophysics*, **4**, 353 - 364.

Lorenzo, G., Zaritzky, N. & Califano, A. (2008). Modeling Rheological Properties of Low-in-fat O/W emulsions stabilized with Xanthan/guar mixtures. *Food Research International.* 41:452-494.

Manera, J., Brotons, J.M., Conesa, A. & Porras, I. (2012). Relationship between air temperature and degreening of lemon (C*itrus lemon* L. Burm. f.) peel color during maturation. *Australian Journal of Crop Science*, **6**(6), 1051 - 1058.

Maskus, H. (2010). Pulse Processing, Functionality and Application. Winnipeg: Manitoba.

Mathur, V. & Mathur, N.K. (2005). Fenugreek and other lesser known legume galactomannans: scope for developments. *Journal of Scientific and Industrial Research,* **64**, 475 - 481.

Mezger, T.G. (2006). The rheology handbook for users of rotational and oscillatory rheometers. 2nd ed., Hannover: Vincentz Network.

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

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

Murevanhema, Y.Y. (2012). Evaluation of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk fermented with lactic acid bacteria as a probiotic beverage. Masters of Technology (Food Technology) Thesis. Cape Peninsula University of Technology, South Africa.

Murevanhema, Y.Y. & Jideani, V.A. (2013). Potential of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk as a probiotic beverage: A review. *Critical Reviews in Food Science and Nutrition,* **53**(9), 954 - 967.

Mwaikambo, L.Y. (2006). Review of the history, properties and application of plant fibres. *African Journal of Science and Technology*, **7**, 120 - 133.

Nikovska, K. (2010). Oxidative stability and rheological properties of oil-in-water Emulsions with Walnut Oil. *Advance Journal of Food Science and Technology* 2: 172-177.

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**(11), 2234 - 2242.

Ojimelukwe, P.C. (2009). Sourcing and Processing of Legumes. In: *Nigerian Agro Raw Materials Development*. (Edited by P. A. Onwualu, S. C. Obasi & U. J. Ukpabi). Abuja: Raw Materials Research and Development Council.

Parrott, M.E. & Thrall, B.E. (1978). Functional properties of various fibres: Physical properties*. Journal of Food Science*, **43**, 759 - 763.

Pfoertner, H. F. & Fischer, J. (2001). Dietary fibres of lupins and other grain legumes. Pages In: *Advanced Dietary Fibre Technology*. (B. V. McCleary & L. Prosky, eds). Pp. 361 – 366. Oxford: Blackwell Science.

Piteira, M.F., Maia J.M., Raymundo, A. & Sousa, I. (2006). Extensional flow behaviour of natural fibre-filled dough and its relationship with structure and properties. *Journal of Non-Newtonian Fluid Mechanics*, **137**, 72 - 80.

Prakongpan, T., Nitithamyong, A. & Luangpituksa, P. (2002). Extraction and application of dietary fibre and cellulose from pineapple cores. *Journal of Food Science*, **67**(4), 1308 - 1313.

Rakha, A. (2011). Characterisation of Dietary Fibre in Cereal Grains and Products. PhD: Natural resources and Agricultural Sciences Thesis, Swedish University of Agricultural Sciences.

Ramanen P, Penttila PA, Svedstrom K, Maunu SL, Serimaa R. (2012). The effect of drying method on the properties and nanoscale structure of cellulose whiskers. *Cellulose*, **19**(3), 901 - 912.

Ramirez-Santiago, C., Ramos-Solis, L., Lobato-Calleros, C., Peña-Valdivia, C., Vernon-Carter, E.J. & Alvarez-Ramírez, J. (2010). Enrichment of stirred yogurt with soluble dietary fibre from Pachyrhizus erosus L. Urban: Effect on syneresis, microstructure and rheological properties. *Journal of Food Engineering*, **101**, 229 - 235.

Rasper, V.F. (1993). Dough rheology and physical testing of dough. In: *Advances in Baking technology.* (Edited by B.S. Kamel and C.E. Stauffer). Pp. 107 - 129. New York: VCH Publishers.

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.

Saeed, F., Pasha, I., Anjum, F.M, & Sultan. M.T. (2011). Arabinoxylans and arabinogalactans: A comprehensive treatise. *Critical Reviews in Food Science and Nutrition*, **51**(5), 467 - 476.

Saha, D. & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: A critical review. *Journal of Food Science and Technology,* **47**(6), 587 – 597.

Sahin, S. & Sumnu, S.G. (2006). Rheological properties of foods. In: *Physical properties of Foods*. Pp. 39 - 105. Turkey: Springer.

Saura-Calixto, F.D. & Bravo, L. (2001). Dietary fibre-associated compounds: Chemistry, analysis, and nutritional effects of polyphenols. *Food Science*, **113**, 415 - 433.

Sharma, A. (2005). Understanding Color Management. In: *Color Management*: *IPA Bulletin.* Pp. 16 – 21. USA: Thomson Delmar Learning.

Silveira, J.L.M. & Bresolin, T.M.B. (2011). Pharmaceutical use of galactomannans. *Quimica Nova*, **34**(2), 292 - 299.

Sivam, A.S., Sun-Waterhouse, D., Quek, S,Y. & Perera, C.O. (2010). Properties of Bread Dough with Added Fibre Polysaccharides and Phenolic Antioxidants: A Review. *Journal of Food Science,* **75**(8), 163 – 174.

Slavin, J. (2013). Fibre and Prebiotics: Mechanisms and Health Benefits. *Nutrients*, **5**(4), 1417–1435.

Soukoulis, C., Lebesi, D. & Tzia, C. (2009). Enrichment of ice cream with dietary fibre: effects on rheological properties, ice crystallization and glass transition phenomena. *Food Chemistry,* **115**, 665 - 671.

Tarrega, A., Duran, L. & Castell, E. (2004). Flow behavior of semi-solid dairy desserts, Effect of temperature. *International Dairy Journal,* **14**, 345-353.

Tcholakova, S., Denkov, N.D. & Danner, T. (2004). Role of surfactant type and concentration for the mean drop size during emulsification in turbulent flow. *Langmuir*, **20**, 7444-7458.

Therdthai, N., Zhou, W. & Adamczak, T. (2002). Optimisation of the temperature profile in bread baking. *Journal of Food Engineering*, **55**, 41 - 48.

Tiwari, U. & Cummins, E. (2011). Functional and physicochemical properties of legume fibres. In: *Pulse Foods: Processing, Quality and Nutraceutical Applications*. 1st ed. (Edited by B.K. Tiwari, A. Gowen. & B. McKenna). Pp. 139, 144. London: Academic Press.

Tomas, M., Latorre, G., Senti, M. & Marrugat, J. (2004). The antioxidant function of high density lipoproteins: A new paradigm in atherosclerosis. *Revista Espanola de Cardiologila,* **57**(6), 557 – 569.

Tosh, S.M. & Yada, S. (2010). Dietary fibres in pulse seeds and fractions: Characterisation, functional attributes and applications. *Food Research International,* **43**(2), 450 - 460.

Tsimikas, S. (2006). Lipoproteins and oxidation. In: *Antioxidants and Cardiovascular Disease.* 2nd ed. (Edited by M.G. Bourassa & J.C. Tardif). Pp 17. Montreal: Springer.

Tudorica, C.M., Kuri, V. & Brennan, C.S. (2002). Nutritional and physicochemical characteristics of dietary fibre enriched pasta. *Journal of Agricultural and Food Chemistry,* **50**(2), 347 - 356.

Vazquez, J.A., Rodriguez-Amado, I., Montemayor, M.I., Fraguas, J., Gonzalez, M.D.P. & Murado, M.A. (2013). Chondroitin Sulfate, Hyaluronic Acid and Chitin/Chitosan Production Using Marine Waste Sources: Characteristics, Applications and Eco-Friendly Processes: A Review. *Marine Drugs*, **11**(3), 747 - 774.

Urriola, P.E., Cervantes-Pahm, S.K. & Stein. H.H. (2013). Fibre in swine nutrition. In: *Sustainable swine nutrition*. 1st ed. (Edited by L.I. Chiba). Pp. 259. West Sussex: Wiley-Blackwell.

Wadouachi, A. & Kovensky, J. (2011). Synthesis of glycosides of glucuronic, galacturonic and mannuronic acids: An overview. *Molecules,* **16**, 3933 – 3968.

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

Wang, N. & Toews. R. (2011). Certain physicochemical and functional properties of fibre fractions from pulses. *Food Research International,* **44**(8), 2515 – 2523.

Wang, J., Jia, F. & Yang, G. (2013). Changes in particle size distribution and solubilisation of casein protein during enzymatic hydrolysis. *Focusing on Modern Food Industry,* **2**(4), 155 – 160.

Wood, J.A. & Grusak, M.A. (2007). Nutritional value of chickpea. In: *Chickpea breeding and management*. (Edited by S.S. Yadav, R. Redden, W. Chen & B. Sharma). Pp.119. Trowbridge: Cromwell Press.

Yang, H., Yan, R., Chen, H. & Lee, D.H. (2007). Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel,* **86**, 1781 - 1788.

Yangilar, F. (2013). The Application of Dietary Fibre in Food Industry: Structural Features, Effects on Health and Definition, Obtaining and Analysis of Dietary Fibre: A Review. *Journal of Food and Nutrition Research,* **3**, 13-23.

Zhang, J & Wang, Z.W. (2013). Soluble dietary fibre from Canna edulis Ker by-product and its physicochemical properties. *Carbohydrate Polymers,* **92**, 289 - 296.

CHAPTER FOUR

PREBIOTIC CHARACTERISTICS OF BAMBARA GROUNDNUT SOLUBLE FIBRES USING THE BATCH FERMENTATION SYSTEM

Abstract

The prebiotic properties of black-eye, brown-eye, brown and red Bambara groundnut (BGN) soluble dietary fibres (SDFs) were assessed using batch culture fermentation systems, monitoring short chain fatty acids (SCFAs) as products of fermentation. The growth rates of *Bifidobacterium bifidum, Bifidobacterium animalis* subsp. *animalis* and *Bifidobacterium breve* in the presence of BGN SDFs were assessed using optical densities (ODs) and microscopic counts. Bacterial growth curves were modelled using the Gompertz kinetic equation. All four BGN SDFs supported the growth of the *Bifidobacterium* spp, confirming their prebiotic properties. The growth of *B. breve* was supported by black-eye SDF > brown-eye SDF > brown SDF > red SDF. The growth of *B. bifidum* was supported by brown-eye SDF > black-eye SDF > red SDF > brown SDF. The growth of *B. animalis* subsp. *animalis* was supported by black-eye SDF > brown-eye SDF > brown SDF > red SDF. The growth rate of *B. breve* ranged from 0.33 Δ log cfu/mL/h (brown SDF) to 0.46 Δ log cfu/mL/h (brown-eye SDF). *B. bifidum* had the highest and least growth rates in red SDF (1.75 Δ log cfu/mL/h) and black-eye SDF (0.46 Δ log cfu/mL/h). *B. animalis* subsp. a*nimalis* had the highest and least growth rates in the presence of red SDF (1.75 Δ log cfu/mL/h) and black-eye SDF (0.54 Δ log cfu/mL/h). The growth rate of *B. animalis* subspp. a*nimalis* as assessed by OD experiments was in the range 0.076 OD units/h (red SDF) to 0.083 OD units/h (black-eye). *B. bifidum* showed the highest growth rate in the presence of brown-eye SDF (0.098 OD units/h) and the least growth rate in the presence of brown SDF (0.084 OD units/h). The growth rate of *B. breve* was in the range 0.082 OD units/h (red SDF) to 0.090 OD units/h (black-eye SDF). The growth rate of *B. breve* in brown SDF and brown-eye SDF were significantly similar ($p > 0.05$). The fermentation of BGN SDFs by *Bifidobacterium* spp produced short chain fatty acids (SCFAs). *B. bifidum* showed preference for black-eye SDF producing 2.91 mMol propionic acid. Acetic acid was produced the most from the fermentation of brown-eye SDF (2.13 mMol) by *B. animalis* subsp. *animalis* and the least from brown SDF fermentation by all three microorganisms. Fructooligosaccharides (FOS) were utilised the most by all microorganisms. The control showed peaks of propionic and acetic acids below the limit of quantification (LOQ) in all microorganisms indicating that BGN SDFs were responsible for the SCFAs produced. Therefore, the bifidogenic nature of BGN SDFs suggests that their inclusion in food systems would impart prebiotic properties and hence increase the market value of that product.

4.1 Introduction

The addition of prebiotics to a variety of food products has become a common occurrence in recent years (Collins & Gibson, 1999). FAO (2001) defines a prebiotic as a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the intestinal microflora that bestows benefits upon the host's health. The concept of prebiotics has received a lot of scientific and industrial interest since its introduction (Cummins *et al*., 2001; FAO, 2001; Gibson *et al*., 2004). To classify a food component as a prebiotic, it must meet three criteria. These criteria are: 1) resistance to gastric acidity, hydrolysis by mammalian enzymes and to gastrointestinal absorption, 2) fermentation by intestinal microflora, and 3) selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well-being (FAO, 2001; Probert *et al.*, 2004; Gibson *et al*., 2004; Roberfroid, 2007).

Various food components such as dietary fibre (DF) have been reported to have prebiotic properties (Manderson *et al.*, 2005). Dietary fibres promote the growth of health promoting microorganisms in the colon. In addition, the fermentation of DFs could lead to the suppression of pathogenic bacteria such as *Salmonella* (Martin-Pelaez *et al*., 2008). Some of the factors that affect the rate of fermentation of DFs include the degree of polymerisation, monosaccharide composition, linkage between monosaccharides, solubility and chain length. Shorter DFs are fermented faster than longer and branched ones (Goderska *et al.*, 2008; Gietl *et al.*, 2012).

The prebiotic effect of a substrate can be determined either *in vivo* or *in vitro* (Rycroft *et al*., 2001; Agarwal *et al.*, 2008; Hur *et al*., 2011). *In vivo* studies are carried out through appropriate nutritional intervention trials in humans, livestock or companion animals using validated methodologies to produce comprehensive scientific data (Roberfroid, 2007). *In vitro* studies make use of batch or continuous culture fermentation systems (Manderson *et al*., 2005; Suharti *et al.*, 2011). These fermentation systems attempt to reproduce physical, anatomical and nutritional characteristics of gastrointestinal regions (Probert *et al*., 2004; Manderson *et al*., 2005; Roberfroid, 2007). Batch-culture vessels can be inoculated with pure cultures of selected species of bacteria and the carbohydrate to be studied (Manderson *et al*., 2005). Roberfroid (2007) described molecular-based procedures used to monitor the stimulation of bacterial activity, production of organic acids, gases and enzymes. These methodologies include short chain fatty acids (SCFAs) analysis, direct community analysis, denaturing/temperature gradient gel electrophoresis and the use of fluorescence in situ hybridization (Probert *et al*., 2004; Roberfroid, 2007; Martin-Pelaez *et al*., 2008, Gietl *et al.*, 2012). These methods have advantages over culture-based technologies as they have improved reliability (Moore, 2011). Short chain fatty acid production using Gas chromatography was assessed in this study. *In vivo,* SCFAs are absorbed by the colonic mucosa and therefore do not accumulate in the colon. However, in static batch fermentation

systems, SCFAs accumulate and reduce the pH to a level that could inhibit the growth of probiotics. To prevent this undesirable condition, the buffer concentration of batch culture media is increased such that the pH remains above pH 5 (Gietl *et al.*, 2012).

Bifidobacterium are Gram positive, non-spore formers that inhabit the gastrointestinal tract and mouth of mammals, including humans (Vlkova *et al.*, 2002; Mahlen & Clarridge, 2009). These bacteria are ubiquitous, obligate anaerobes and lack flagella (Kneifel *et al.,* 2000; Baffoni *et al*., 2013). They are saccharolytic as they are capable of fermenting a wide array of saccharides (Cummings *et al*., 2001; Janda & Abbott, 2002; Korakli *et al.*, 2002; Collado & Hernandez, 2007). Several media are used for the culturing and enumeration of *Bifidobacterium*. These include de Man, Rogosa, Sharpe (MRS) media which is preferable for pure cultures and Reinforced Clostridial Prussian Blue (RCPB) media (Moriya *et al*., 2006; Fernando *et al.*, 2011). The effect of BGN SDFs on the growth of *Bifidobacterium* spp. over time was described using growth curves. To describe bacterial growth curves, several sigmoid functions such as Gompertz, Stannard, Richard, Schnute and Logistic models can be used (Lu *et al.*, 2006). However, Gompertz function has been reported to be the most used and most effective in predictive food microbiology (Ye *et al*., 2013).

Although research on the prebiotic effects of DF from various sources has been conducted, the prebiotic characteristics of BGN soluble dietary fibres (SDFs) have not been reported. Hence, this research aimed to investigate the prebiotic properties of BGN SDFs employing pure cultures of *Bifidobacterium* spp as probiotics.

4.2 Materials and Methods

4.2.1 Source of BGN, cultures and chemicals

Freeze dried pure cultures of *Bifidobacterium animalis* subsp. *animalis* ATCC 25527*, Bifidobacterium bifidum* ATCC 11863 and *Bifidobacterium breve* ATCC 15700 were purchased from Davies Diagnostics (Pty) Ltd, Randburg, South Africa. BGN SDFs (black-eye, brown-eye, brown and red) were extracted using the wet milling method as described in Chapter 3 section 3.2 (page 54). All the chemicals used in this study were of analytical grade and were purchased from Sigma-Aldrich®, Gauteng, South Africa unless otherwise stated. Figure 4.1 gives an overview of the analyses carried out in this chapter. Serial dilutions were made in ¼ strength Ringer's solution (Quantum Biotechnologies, Johannesburg, South Africa).

4.2.2 Culture validation and subculturing

The three freeze dried *Bifidobacterium* cultures were kept refrigerated at 5°C until the time of the experiments, when they were activated. To activate each microorganism, one pellet was aseptically removed from the Lyfo disk$^{(R)}$ vial (Davies Diagnostics (Pty) Ltd, Randburg, South Africa) and homogenised in 0.5 mL of sterile water.

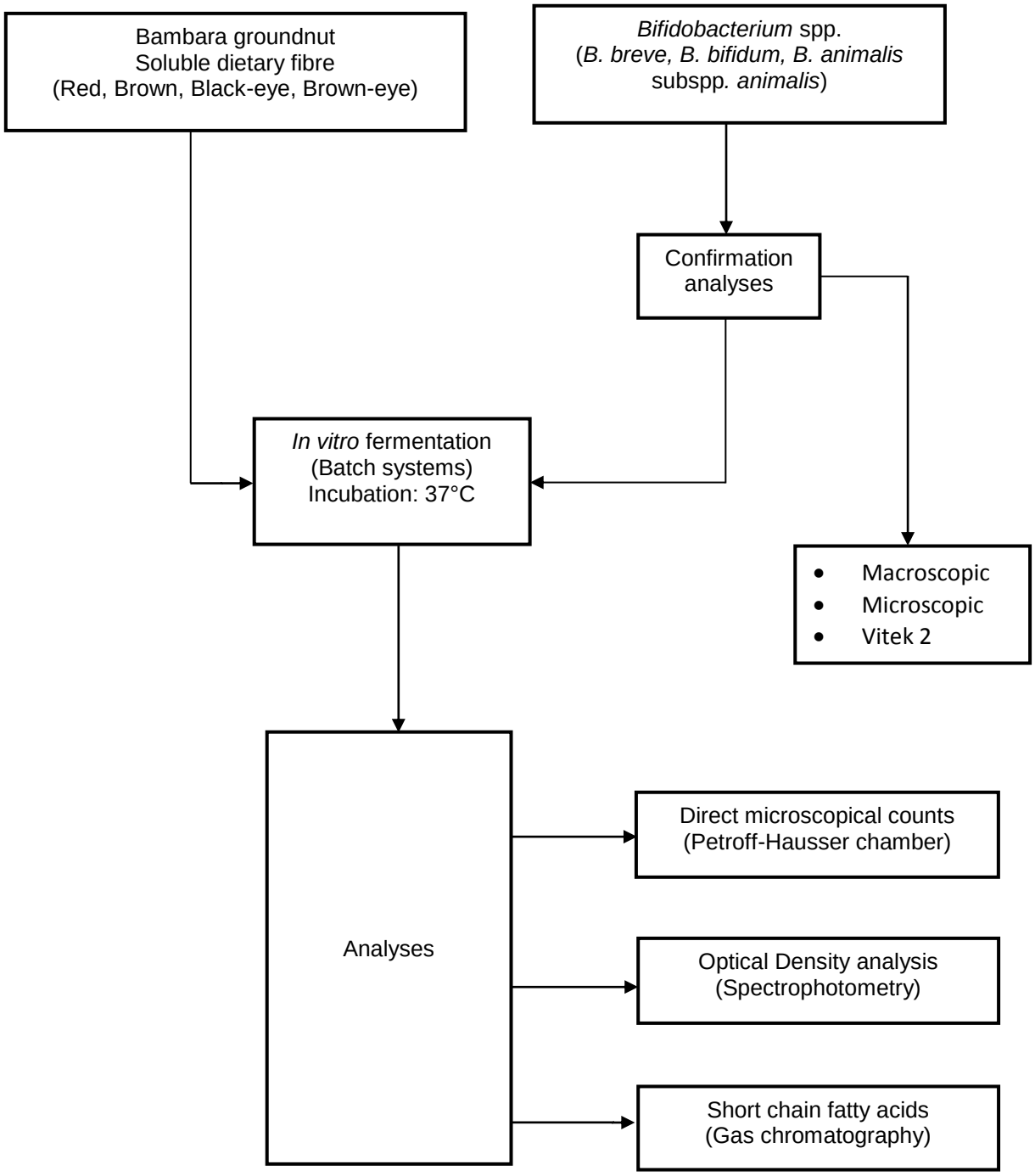

Figure 4.1 Experimental design for Chapter 4.

The microbial suspension was then streaked on MRS agar medium (BioLab, BioLab Diagnostics, Midrand, South Africa) and incubated at 37°C for 24 h in an anaerobic jar in the presence of an Oxoid^{TM} anaerobic gas generating kit (Quantum Biotechnologies, Johannesburg, South Africa) and an Oxoid^{TM} anaerobic indicator (BR0055). Following incubation, macroscopic and microscopic observations were done on single colonies of each microorganism. Confirmation of the cultures was done using the Vitek 2 system (bioMerieux Inc., North Carolina, USA).

1. *Vitek automated microbial identification system*

Vitek 2 system microbial identification was carried out on 24 h *Bifidobacterium* spp. cultures following the method of Lee *et al.* (2011). Colonies of each culture were mixed with 3 mL sterile saline (0.45% Sodium chloride) in plastic tubes to a turbidity of 3.8 McFarland standards (McF) using a calibrated Vitek 2 Densichek plus instrument (bioMerieux Inc., North Carolina, USA). Vitek$^{(R)}$ ANC ID cards were then inserted into the plastic tubes held in a cassette. The cassette was inserted into the Vitek$^{(R)}$ 2 instrument (bioMerieux, South Africa) and analysis proceeded. Supplementary analyses considered Gram characteristics, morphology as well as aerotolerance of the microorgamins.

4.2.3 Assessment of the growth rates of *Bifidobacterium* spp.

A 3 x 4 level factorial design consisting of probiotics (*B. breve, B. bifidum* and *B. animalis* subspp. *animalis*) at 3 levels and BGN SDFs at 4 levels (black-eye, brown-eye, brown and red) was used, with each design point in triplicate. Optical density measurements and direct microscopic counts were used in the assessment of the growth rates of *Bifidobacterium* spp. Growths of *Bifidobacterium* spp. in broth without any BGN SDF added were used as controls. BGN SDFs were pasterurised at 70°C for 15 min prior to use to avoid contamination.
The following sections detail the procedures of the experiments.

1. *Optical density measurements of Bifidobacterium spp*

A modified method of Martin-Pelaez *et al*. (2008) was adopted in this study. Plates of MRS agar were streaked from the stock cultures and incubated for 24 h at 37°C under anaerobic conditions. McCartney bottles containing 10 mL MRS broth (BioLab, BioLab Diagnostics, Midrand South Africa), were then inoculated with one colony from each plate and incubated overnight at 37°C. After incubation, each bacterial cell suspension (0.1 mL) was inoculated into a McCartney bottle containing 1 mL of each BGN SDF, separately (1% w/w) and 9 mL glucose free MRS medium. Tubes were then further incubated for 24 h at 37°C. The optical density (OD) of each culture mixture (1.5 mL) was determined at 4 h intervals for up to 24 h at a wavelength of 660 nm using a temperature (20°C) controlled spectrophotometer (UV-1700 PharmsSpec, Shimadzu, Japan). Plastic cuvettes (Macro PS, Lasec, 10×10×45 mm)

were used. Subsequently, the growth rate expressed in OD units/h of the bacterial strains in each BGN SDF-containing medium was calculated.

2. *Direct microscopic counts of Bifidobacterium spp*

A method described by Brock *et al*. (1994) was adopted in this study. McCartney bottles containing 9 mL glucose free MRS broth (BioLab, BioLab Diagnostics, Midrand South Africa) and 1 mL of each BGN SDF (1% w/w), separately were inoculated with 0.1 mL 24 h culture suspension. Tubes were then incubated for 24 h at 37°C. At 4 h intervals, 1 mL samples were drawn from the stock culture and diluted appropriately. The number of cells in a population of each culture was determined by direct microscopic count (Olympus microscope CX21 Model CX21FS1, Olympus Corporation, Tokyo, Japan) using a Petroff-Hausser counting chamber with a depth of 0.02 mm (Hausser Scientific, Philadelphia, USA). Samples (10 µL) were carefully pipetted under a cover glass (0.25 mm thickness). The number of cells per unit area on the grid was counted giving a measure of the number of cells per small chamber volume. The calculation of the number of microorganisms was according to equation 4.1 and the results were expressed as log colony forming units per mL (cfu/mL).

$$N \times 25 \times 50 \times 1000 \times \text{dilution factor} \qquad \textit{Equation 4.1}$$

Where N is the number of cells, 25 is the number of squares in the grid, 50 is the depth of the grid, 1000 is the conversion factor to mL.

3. *Modelling of Bifidobacterium spp. growth curves*

The data were analysed by fitting the bacterial growth curves to Gompertz equation (Equation 4.2) as modified by Zwietering *et al.* (1990) (Guerzoni *et al.*, 1996; Corbo *et al.,* 2005).

$$y = k + A \times \exp\{-\exp\left[\left(\mu_{max} \times \frac{e}{A}\right) \times (\lambda - t) + 1\right]\} \qquad \textit{Equation 4.2}$$

Where y = log cfu/mL of probiotics, k = initial microbial load, t = time t, A = the increase in log cfu/g between time = 0 and the maximum cell count at the stationary phase (log cfu/g), μ_{max} = maximum growth rate (Δ log cfu/g per day), λ = length of lag phase expressed in hours, e is Euler's number with a value of 2.7182.

Constrained nonlinear regression analysis (IBM SPSS, version 22, 2013) was used to estimate the model parameters. The goodness-of-fit of the models was assessed using the coefficient of determination (R^2).

4.2.4 *In vitro* batch culture fermentations

The method of Gietl *et al.* (2012) was followed in this study with modifications. The fermentation experiments were carried out in order to assess the fermentation pattern of BGN SDFs as well as to evaluate the effect of fermentation on the *Bifidobacterium* spp. A 3 x 5 factorial design was carried out with *Bifidobacterium* spp. (*B. breve*, *B. bifidum*, *B. animalis* subspp. *animalis*) and saccharides (black-eye, brown-eye, brown, red and fructooligosaccharides), with each design point conducted in triplicate. SCFA production by *Bifidobacterium* spp. without any saccharide were used as controls. Following this design, a basal nutrient medium (18 mL) was dispensed into each McCartney bottle. The medium was made up of the following: peptone water (0.78 g/L) (Quantum Biotechnologies, Johannesburg, South Africa), NaCl (0.49 g/L), KH_2PO_4 (1.6 g/L), bile salts (sodium glycocholate and sodium taurocholate) (0.4 g/L), L-cysteine hydrochloride (0.5 g/L), K_2HPO_4 (0.5 g/L), $MgSO_4.7H_2O$ (0.01 g/L), $NaHCO_3$ (7.1 g/L), $CaCl_2.6H_2O$ (0.007 g/L), haemin (0.5 g/L), yeast extracts (2 g/L) (Quantum Biotechnologies, Johannesburg, South Africa), Tween 80 (2 mL/L), vitamin K1 (10 µL/L). Resazurin solution (4 mL/L) was used as an indicator of anaerobiocity. The medium was autoclaved (121°C for 15 min) prior to use. To each tube, 1 mL of a 24 h bacterial suspension and 1 mL of 10% (w/v) saccharide was added. BGN SDFs were pasterurised at 70°C for 15 min prior to use to avoid contamination. Anaerobic conditions were maintained by incubation in anaerobic gas jars in the presence of anaerobic gas packs. Samples were collected at 0, 5, 10 and 24 h for SCFA analysis (Manderson *et al.*, 2005; Martin-Pelaez *et al*., 2008). Anaerobic gas packs were replaced after each removal of sample to restore anaerobic conditions.

4.2.5 Short-chain fatty acid analysis

Prior to Gas Chromatography (GC) analysis, samples (2 mL) were taken from the McCartney bottles and vortexed for 1 min before centrifugation (13000 x *g*, 5 min) to remove solid materials (Vigsnæs *et al*., 2011). The method of Siedlecka *et al.* (2008) was followed for preparation of samples for GC analysis. The supernatants were acidified to pH 2 using 65% nitric acid then 1 mL samples were shaken along with 1 mL diethyl ether for 5 min. The top layer (ether) was carefully transferred to clean test tubes. Anhydrous sodium sulphate (500 mg) was then added to remove excess moisture. Ether phases (500 µm) were then transferred to 1 mL glass vials and 150 µm of 2% sulphuric acid in methanol was added. The vials were then vortexed and analysed using an Agilent 7890B Gas Chromatograph, dual injector with dual Flame Ionised Detectors (FID) fitted with a CP-Sil 88, SP-2560, BPX-70, 100 m x 250 µm x 0.25 µm capillary column. The injector temperature was set at 250°C, the detector temperature was at 300°C and nitrogen was used as carrier gas. The analyses were performed using a temperature programme of 3 min at 50°C and a gradient from 50°C to 110°C at 5°C/min. An injection volume of 1 µL and a split ratio of 25:1 were used in this

study. Quantification of the samples was carried out using calibration curves of acetic and propionic acids in concentrations between 0.01 mMol and 5.00 mMol and results were expressed in mMol/L (Vigsnæs *at al.*, 2011). The experiments were carried out in triplicate. Chemstation (Agilent Technologies) was used for calibration and calculation of the internal response factor for quantification of peak areas within samples as reported by Hobden *et al*. (2013).

4.2.6 Data analysis

For statistical analysis, IBM Statistical Package for the Social Science (IBM SPSS, version 22, 2013) was used. The results were subjected to Multivariate Analysis of Variance (MANOVA) to determine mean differences between treatments. Duncan's multiple range test was conducted to separate mean differences where differences existed. Values were expressed as mean ± standard deviation. Values were expressed as mean ± standard error for the Gompertz parameters. The goodness-of-fit of Gompertz parameters was assessed using the coefficient of determination (R^2).

4.3 Results and Discussion

4.3.1 Confirmation of *Bifidobacterium* spp.

Bifidobacterium breve colonies appeared relatively larger than those of the other two species, had a pale cream colour and were circular in shape. *B*. *bifidum* colonies were observed as smooth, transculent, cream, small and round. *B. animalis* subsp. *animalis* colonies were observed as shiny, off-white, small and round. All three species were Gram positive rods. Microscopically, *B. breve* exhibited the smallest rods, *B. bifidum* exhibited rods of different shapes and sizes and *B*. *animalis* subsp. *animalis* was observed as small, short rods similar to those of *B. breve*. The observations were in agreement with *Bifidobacterium* description of Baffoni *et al.* (2013) and Rockova *et al.* (2013). The Vitek 2 system confirmed the identity of the *Bifidobacterium* spp.

4.3.2 Growth patterns of *Bifidobacterium* spp.

1. *Optical densities of Bifidobacterium spp*.

Table 4.1 gives the hourly growth rates of each *Bifidobacterium* spp. in the presence of four varieties of BGN SDFs assessed using optical densities. *B. breve* showed the least growth rate in the presence of red SDF (0.082 OD units/h) and the highest ($p < 0.05$) growth rate in the presence of black-eye SDF (0.090 OD units/h). The growth of *B. breve* was supported by black-eye SDF > brown-eye SDF > brown SDF > red SDF (Figure 4.2a). The growth rate of *B. breve* in brown SDF and brown-eye SDF were significantly ($p > 0.05$) similar. After 24 h, the OD of *B. breve* ranged from 2.164 OD units (black-eye SDF) to 1.970 OD units (red SDF) (Figure 4.2a).

Table 4.1 Optical densities for *Bifidobacterium* spp. grown in the presence of Bambara groundnut soluble dietary fibres (OD units/h)

Variety	*B. breve*	*B. bifidum*	*B. animalis* subsp. *animalis*
Black-eye	0.090[a]	0.094[a]	0.083[a]
Brown-eye	0.085[b]	0.098[a]	0.079[b]
Brown	0.083[b]	0.084[b]	0.079[b]
Red	0.082[c]	0.091[c]	0.076[c]
Control	0.070[d]	0.075[d]	0.064[d]

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [p > 0.05] different. OD: optical density.

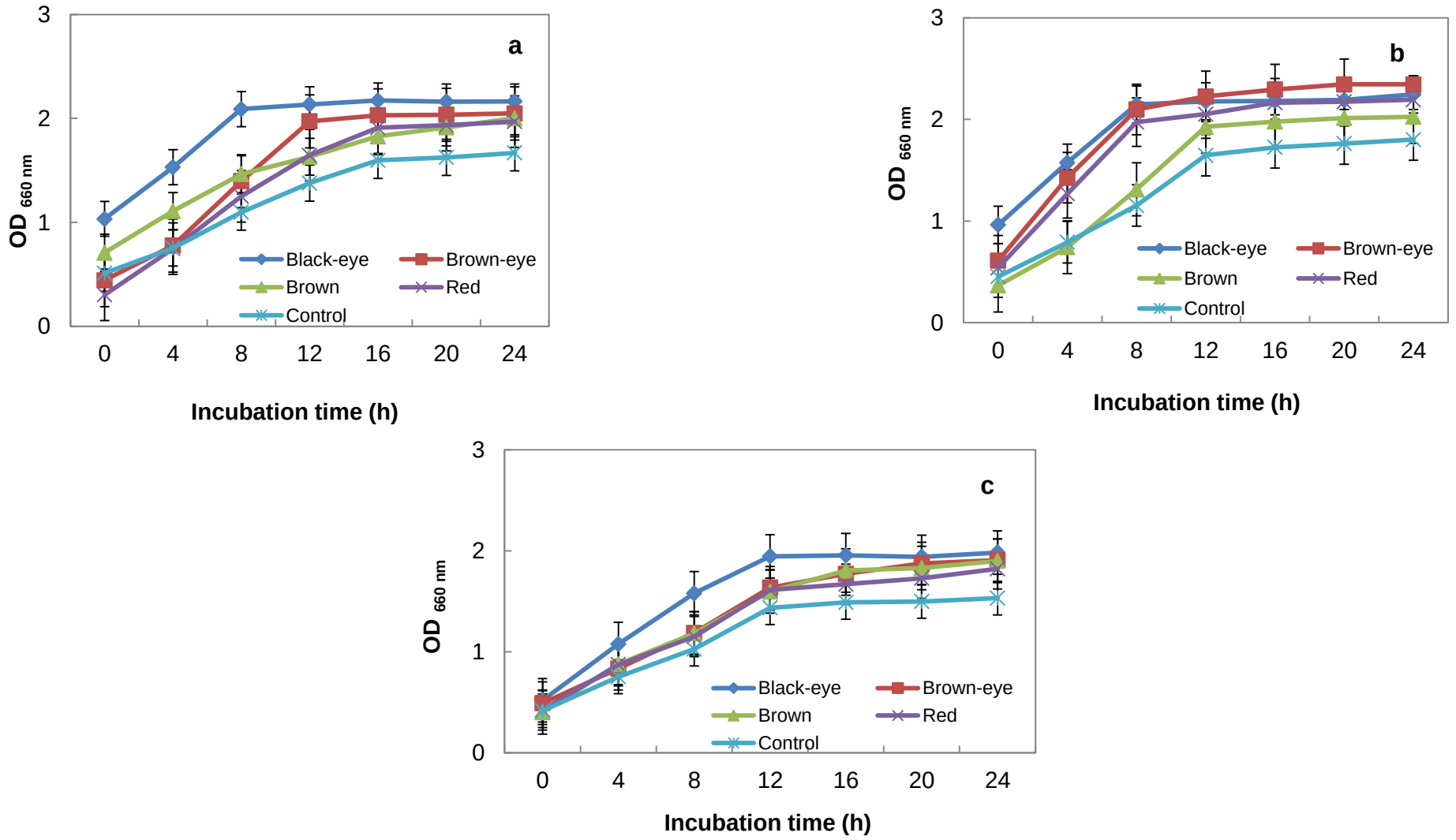

Figure 4.2 Growth curves of *Bifidobacterium* spp. in the presence of four varieties of Bambara groundnut soluble dietary fibres (a) *B. breve* (b) *B. bifidum* (c) *B. animalis* subsp. *animalis.*

B. bifidum showed the highest growth rate in the presence of brown-eye SDF (0.098 OD units/h) and the least growth rate in the presence of brown SDF (0.084 OD units/h). The growth of *B. bifidum* was supported by brown-eye SDF > black-eye SDF > red SDF > brown SDF (Figure 4.2b). The growth rate of *B. bifidum* in black-eye SDF and brown-eye SDF was significantly ($p > 0.05$) similar. The growth pattern of *B. bifidum* observed in this study was similar to that of *B. bifidum* (2 OD units) grown in the presence of human oligosaccharides (Asakuma *et al.*, 2011). After 24 h, the OD of *B. bifidum* ranged from 2.345 OD units (brown-eye SDF) to 2.026 OD units (brown SDF).

B. animalis subspp. a*nimalis* showed the least ($p < 0.05$) growth rate in the presence of red SDF (0.076 OD units/h) and the highest ($p < 0.05$) growth rate in the presence of black-eye SDF (0.083 OD units/h). The growth of *B. animalis* subsp. *animalis* was supported by black-eye SDF > brown-eye SDF > brown SDF > red SDF (Figure 4.2c). The growth rate of *B. animalis* subspp. a*nimalis* in black-eye SDF and brown SDF was significantly ($p > 0.05$) similar. After 24 h, the OD of *B. animalis* subspp. *animalis* ranged from 1.984 OD units (black-eye SDF) to 1.822 OD units (red SDF). All three *Bifidobacterium* spp. showed significantly ($p < 0.05$) low growth rates in the controls (Table 4.1), which indicated that BGN SDFs supported their growth.

Furthermore, the increase in bacterial population in terms of OD of the three *Bifidobacterium* spp. differed significantly ($p < 0.05$). The optical density of microorganisms is assessed as turbidity. Light is passed through a microbial suspension and the amount of light scattered serves as an indication of the biomass present in the suspension (Sutton, 2011). Dietary constituents that stimulate fermentation result in an increase in biomass (Slavin, 2013). The ability of *Bifidobacterium* spp. to utilise SDFs as substrates during fermentation has been reported by several researchers (Fernando *et al.,* 2011; Turroni *et al.*, 2011; Rockova *et al.,* 2013). The shapes of the growth curves of all three *Bifidobacterium* spp. grown in the presence of BGN SDFs were in accordance with the description of Kniefel *et al.* (2000). The researchers reported that most *Bifidobacterium* strains develop their maximum growth capacities followed by a stationary phase within 24 h of incubation.

Higher growth rates of *B. breve* (0.410 OD units/h) and *B. animalis* subspp. *animalis* (0.196 - 0.440 OD units/h) in the presence of raffinose and fructooligosaccharides (FOS) were reported (Trojanova *et al.*, 2006; Martin-Pelaez *et al.,* 2008). Raffinose is a trisaccharide composed of galactose, glucose and fructose and FOS are linear oligosaccharides composed of fructose monomers. Consequently, the shorter chains of FOS and raffinose enable them to be more readily and rapidly fermented than the longer BGN dietary fibres (Henningsson *et al.*, 2001; Toppin & Clifton, 2001; Farooq *et al.*, 2013). In addition, previous studies have suggested that *Bifidobacterium* spp. have a higher affinity for oligosaccharides than for polysaccharides (Kniefel *et al.,* 2000) because the cleavage of oligosaccharides to monomers by *Bifidobacterium* glycosidases occurs more rapidly and

easier than the hydrolysis of polysaccharides to monomers (Palframan *et al.*, 2003). Under similar conditions to this study, Martin-Pelaez *et al.* (2008) reported higher growth rates of *B. breve* than of *B. bifidum* in the presence of FOS while in this study *B. bifidum* showed a higher growth rate in BGN SDFs than *B. breve*. This could be an indication that *B. bifidum* have a higher preference for BGN SDFs than *B. breve* while *B. breve* have a higher preference for FOS than *B. bifidum.* *Bifidobacterium* spp. particularly *B. bifidum* are common in infant faecal waste (Vlkova *et al.*, 2005; Turroni *et al.*, 2012; Amin *et al.*, 2013) and therefore the high preference of *B. bifidum* for BGN SDFs would make these fibres useful ingredients in baby foods.

The fermentability of dietary fibres by *Bifidobacterium* spp could be a confirmation of their prebiotic properties (Kaplan & Hutkins, 2000; Manderson *et al*., 2005). For a fibre to be classified as a prebiotic, it must, among other things, be fermentable by intestinal microflora as well as stimulate the growth and activity of probiotic bacteria such as *Bifidobacterium* (FAO, 2003). Hence, it can be deduced that BGN SDFs are bifidogenic and were responsible for the increased growth rates of *Bifidobacterium* spp, thus making them prebiotic. The inclusion of these SDFs in food systems would impart prebiotic properties to that particular system and consequently increase its market value.

2. *Growth parameters of Bifidobacterium* spp. in BGN SDFs

Direct microscopical counting was used as a supplement to optical density assessment. Figure 4.3 shows the increase in cell counts of *B. breve*, *B. bifidum* and *B. animalis* subsp. *animalis* in the presence of black-eye, brown-eye, brown and red BGN SDFs. *B. breve* and *B. bifidum* showed a preference for brown-eye SDF > black-eye SDF > red SDF > brown SDF (Figures 4.3a and 4.3b). *B. animalis* subsp. *animalis* showed preference for black-eye SDF > brown-eye SDF > brown SDF > red SDF (Figure 4.3c). The preference of *B. bifidum* for BGN SDFs was in agreement with the OD measurements (Figure 4.2b). The log cycle of *B. breve*, *B. bifidum* and *B. animalis* subsp. *animalis* in BGN SDFs ranged from 5.27 log cfu/mL (brown SDF) – 6.67 log cfu/mL (brown-eye SDF), 6.25 log cfu/mL (brown SDF) – 7.85 log cfu/mL and 6.72 log cfu/mL – 7.65 log cfu/mL, respectively. The results of optical density and direct microscopic analyses were in agreement, with BGN SDFs supporting the growth of *Bifidobacterium* spp. in both instances.

In the first few hours of incubation, the growth of all *Bifidobacterium* spp. in BGN SDFs was relatively slow as shown by a lag-like phase in Figure 4.3. This could be attributed to the microorganisms acclimatising to the new environment and still recovering from shock encountered during transfer (Laird *et al.*, 2004; Todar, 2012; Rolfe *et al.*, 2012). Similar growth characteristics of *Bifidobacterium* spp. growing in the presence of wheat germs were reported by Barascu *et al.* (2007).

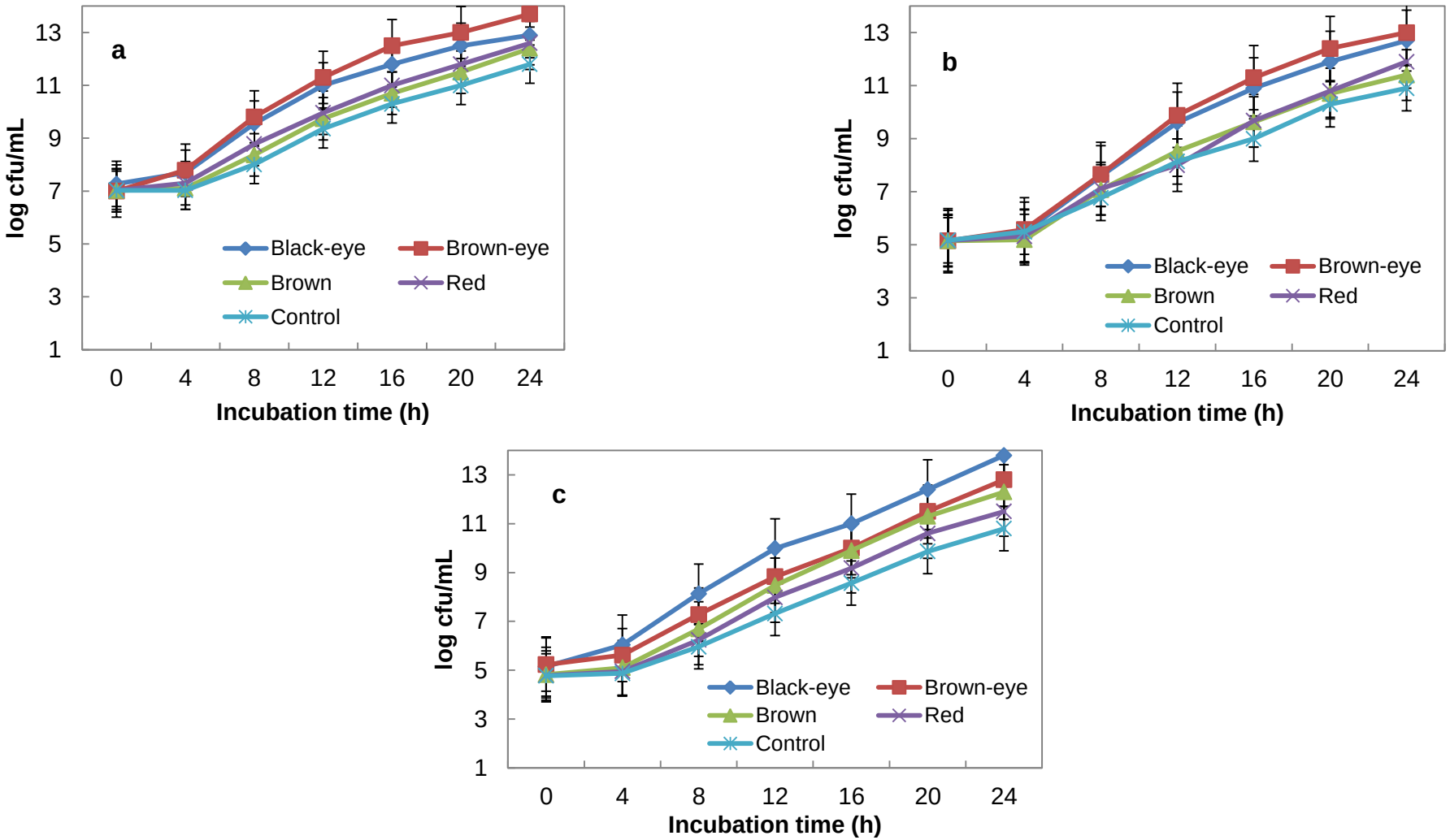

Figure 4.3 Microscopic counts of *Bifidobacterium* spp. in the presence of four varieties of Bambara groundnut soluble dietary fibres (a) *B. breve* (b) *B. bifidum* (c) *B. animalis* subsp. *animalis.*

The Gompertz equation was suitable for describing the growth of *Bifidobacterium* spp. in SDFs. The coefficient of determination (R^2) of all three *Bifidobacterium* spp. growing in the presence of the four BGN SDFs was above 0.900, with the exception of *B. bifidum* in the presence of red and brown SDFs as well as *B. animalis* subsp. *animalis* in the presence of brown SDF. Hence, Gompertz model could be accurately employed in predicting the individual responses of the probiotics to BGN SDFs. Table 4.2 gives the Gompertz parameters. The growth rate of *B. breve* was highest in the presence of brown-eye SDF (0.46 Δ log cfu/mL/h) and least in the presence of brown SDF (0.33 Δ log cfu/mL/h) (Table 4.2). The initial cell concentration (*k*) of *B. breve* was in the range 6.77 log cfu/mL (brown-eye SDF) to 7.17 log cfu/mL (black-eye SDF). *B. breve* showed the highest and the least maximum cell count increase at the stationary phase (*A*) in brown-eye SDF (7.15 log cfu/mL) and in black-eye SDF (5.79 log cfu/mL), respectively. *B. breve* showed the least lag phase in the presence of brown-eye SDF (1.71 h) and the longest in brown SDF (3.69 h). The growth rate of *B. breve* was slowest in the control (0.32 Δ log cfu/mL/h) than in all four BGN SDFs. Therefore, it can be deduced that BGN SDFs supported the growth of *B. breve.*

B. bifidum had the highest and least growth rates in red SDF (1.75 log Δ cfu/mL/h) and black-eye SDF (0.46 Δ log cfu/mL/h) (Table 4.2). The initial cell concentration (*k)* of *B. bifidum* was in the range 4.99 log cfu/mL (black-eye SDF) to 5.24 log cfu/mL (red SDF). The least maximum cell count increase at the stationary phase (*A*) of *B. bifidum* ranged from 4.19 log cfu/mL (brown SDF) to 8.30 log cfu/mL (brown-eye SDF). *B. bifidum* showed the shortest lag phase in black-eye SDF (3.41 h). The growth rate of *B. bifidum* was slowest in the control (0.33 Δ log cfu/mL/h) than in all four BGN SDFs. Hence, it can be concluded that BGN SDFs supported the growth of *B. bifidum.*

B. animalis subsp. a*nimalis* had the highest and least growth rates in the presence of red SDF (1.75 Δ log cfu/mL/h) and black-eye SDF (0.54 Δ log cfu/mL/h). The initial cell concentration (*k*) of *B. animalis* subsp. *animalis* was in the range 3.68 log cfu/mL (black-eye SDF) to 5.99 log cfu/mL (brown SDF). The maximum cell count increase at the stationary phase (*A*) of *B. animalis* subsp. *animalis* ranged from 7.00 log cfu/mL (brown SDF) to 12.85 log cfu/mL (black-eye SDF). The growth rate of *B. animalis* subsp. *animalis* was slowest in the control (0.36 Δ log cfu/mL/h) than in BGN SDFs thus proving the ability of BGN SDFs to support the growth of *B. animalis* subsp. *animalis.*

B. animalis subsp. *animalis* had the shortest lag phase in the presence of brown-eye SDF (1.83 h) and the longest in the presence of brown SDF (7.30 h). The growth rates of the three *Bifidobacterium* spp. were slowest ($p < 0.05$) in the absence of BGN SDFs (controls) thereby confirming the ability of BGN SDFs to support their growth. The growth rates of *B. breve, B. bifidum* and *B. animalis* subsp*. animalis* in the controls were 0.32 Δ log cfu/mL/h, 0.33 Δ log cfu/mL/h and 0.36 Δ log cfu/mL/h, respectively.

Table 4.2 Gompertz parameters for the influence of Bambara groundnut soluble dietary fibres on the growth of *Bifidobacterium* spp.

***Bifidobacterium* spp.**	**Black-eye**	**Brown-eye**	**Brown**	**Red**	**Control**
B. breve					
A	5.79 ± 0.14	7.15 ± 0.23	6.29 ± 0.34	6.47 ± 0.30	5.37 ± 0.23
K	7.17 ± 0.09	6.77 ± 0.14	6.85 ± 0.11	6.82 ± 0.12	6.92 ± 0.08
μ_{max}	0.44 ± 0.02	0.46 ± 0.02	0.33 ± 0.02	0.34 ± 0.01	0.32 ± 0.01
Λ	2.83 ± 0.36	1.71 ± 0.50	3.69 ± 0.63	2.65 ± 0.60	4.74 ± 0.48
R^2	0.997	0.997	0.995	0.997	0.996
B. bifidum					
A	8.11 ± 0.23	8.30 ± 0.13	4.19 ± 1.49	6.21 ± 1.42	7.10 ± 0.70
K	4.99 ± 0.11	5.05 ± 0.06	5.15 ± 1.05	5.24 ± 0.58	4.96 ± 0.23
μ_{max}	0.54 ± 0.02	0.58 ± 0.01	0.67 ± 1.05	1.75 ± 9.00	0.33 ± 0.02
Λ	3.41 ± 0.40	3.64 ± 0.21	5.21 ± 5.80	13.32 ± 13.80	2.78 ± 1.19
R^2	0.997	0.999	0.416	0.715	0.989
B. animalis					
A	12.85 ± 1.96	11.04 ± 1.07	7.00 ± 2.12	8.31 ± 0.36	7.82 ± 0.30
K	3.68 ± 0.90	4.68 ± 0.28	5.99 ± 0.58	4.63 ± 0.10	4.64 ± 0.06
μ_{max}	0.43 ± 0.02	0.39 ± 0.01	0.48 ± 0.17	0.41 ± 0.01	0.36 ± 0.01
Λ	-2.08 ± 2.63	1.83 ± 1.04	7.30 ± 2.87	4.33 ± 0.44	4.82 ± 0.33
R^2	0.994	0.996	0.808	0.998	0.999

Gompertz parameters Where k = initial microbial load (log cfu/mL), A = maximum cell count increase at the stationary phase (log cfu/mL), μ_{max} = maximum growth rate (Δ log cfu/mL/h), λ = length of lag phase expressed in hours. Values are mean ± standard error. R^2: coefficient of determination: (Residual sum of squares / corrected sum of squares).

The initial cell concentration (*k*) of *B. breve, B. bifidum* and *B. animalis* subsp*. animalis* in the controls were 6.92 log cfu/mL, 4.96 log cfu/mL and 4.64 log cfu/mL, respectively. The maximum cell count increase at the stationary phase (*A*) of *B. breve, B. bifidum* and *B. animalis* subsp*. animalis* in the controls were from 5.37 log cfu/mL, 7.10 log cfu/mL and 7.82 log cfu/mL, respectively.

FAO (2001) defines a prebiotic as a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the intestinal microflora. Hence, it can be deduced that BGN SDFs have prebiotic properties as their effect on the growth of *Bifidobacterium* spp. fitted the FAO (2001) prebiotic definition. The application of the Gompertz parameters in modelling of the bacterial growth curve has been successfully applied by several researchers (Guerzoni *et al.*, 1996; Sinigaglia *et al.*, 2003; Corbo *et al.*, 2005; Lu *et al.*, 2006; Slacanac *et al.*, 2013). Gompertz kinetics provided an insight into the growth trends of *Bifidobacterium* spp in BGN SDFs. The application of mathematical modelling in assessing the growth rates of *Bifidobacterium* spp. in the BGN SDFs would help in establishing some scientific basis to solve questions such as the amount of SDF to add in products as well as describe the fermentation of the SDFs in the human colon.

4.3.3 Short chain fatty acids

The production of propionic acid and acetic acid from BGN SDF substrates and FOS fermentation is shown in Figure 4.4 and Figure 4.5, respectively. At the beginning of the experiment, propionic acid was below the limit of quantification (LOQ) for all BGN SDFs as well as FOS. Among BGN SDFs, propionic acid production by *B. breve* ranged from 0.11 mMol (brown SDF) to 2.79 mMol (brown-eye SDF) after the 24 h period. The fermentation of all BGN SDFs by *B. breve* yielded significantly ($p < 0.05$) different amounts of propionic acid. FOS fermentation by *B. breve* yielded a significantly ($p < 0.05$) higher amount of propionic acid (3.53 mMol) after 24 h (Figure 4.4a). Gietl *et al.* (2012) studied the fermentation of a variety of disaccharides and trisaccharides by *B. breve* and reported a propionic acid quantity of 0.32 mMol. Relatively higher quantities of propionic acid produced by fermentation of BGN SDFs of *B. breve* were reported in this study. This indicated that BGN SDFs are more fermentable than some prebiotic disaccharides and trisaccharides. The production of acetic acid from the fermentation of BGN SDFs by *B. breve* ranged from 1.67 mMol (brown-eye SDF) to 0.30 mMol (brown SDF). Acetic acid production from brown-eye and black-eye SDFs by *B. breve* was significantly ($p > 0.05$) similar in the first 5 h, thereafter, more acetic acid was produced from brown-eye SDF fermentation (Figure 4.5). After 24 h, brown-eye SDF fermentation slowed down and an acetic acid content similar ($p > 0.05$) to that of black-eye SDF (1.66 mMol) was produced.

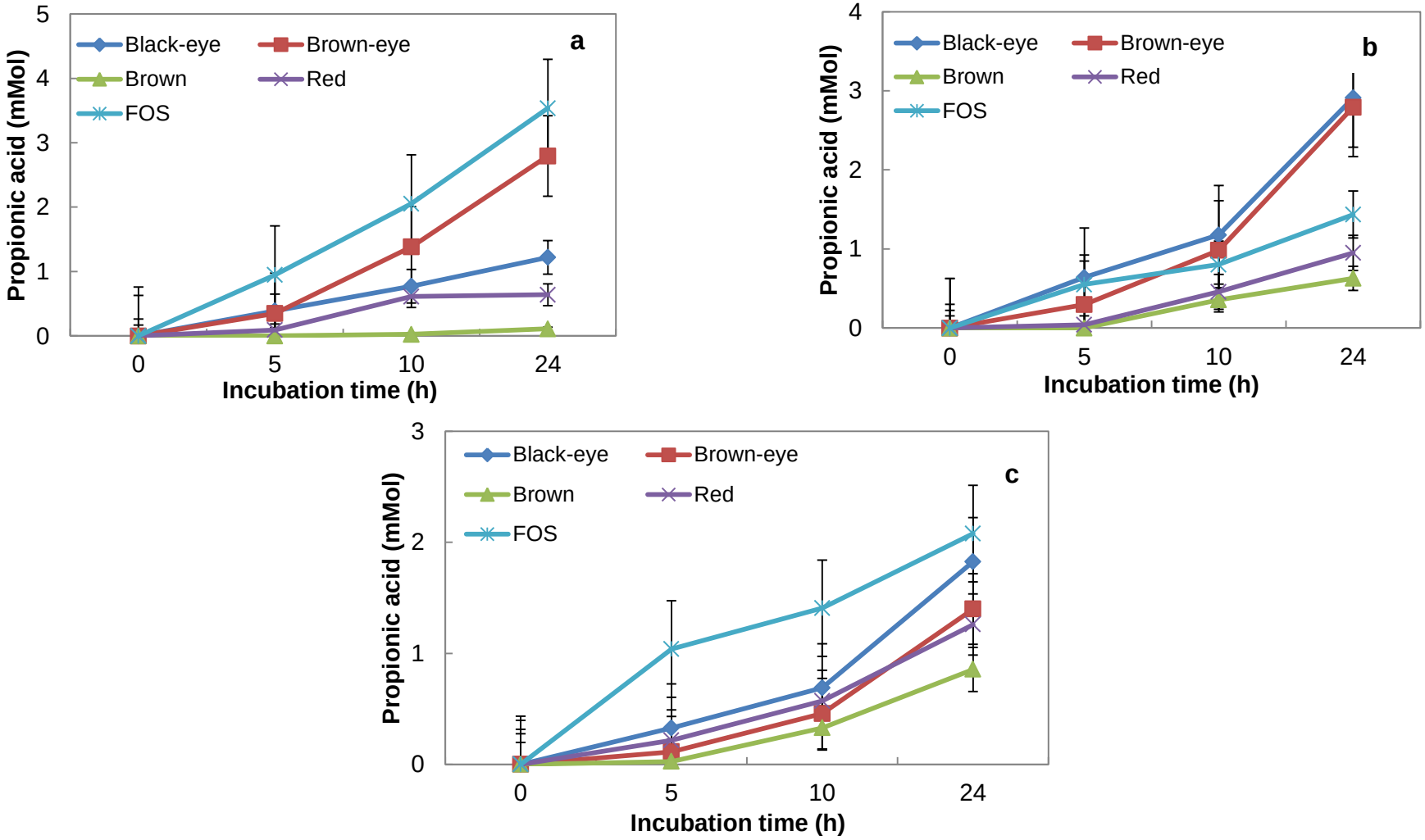

Figure 4.4 Propionic acid production after 24 h batch fermentation of Bambara groundnut soluble fibres and fructooligosaccharides by *Bifidobacterium* spp. (a) *B. breve* (b) *B. bifidum* (c) *B. animalis* subsp. *animalis.*

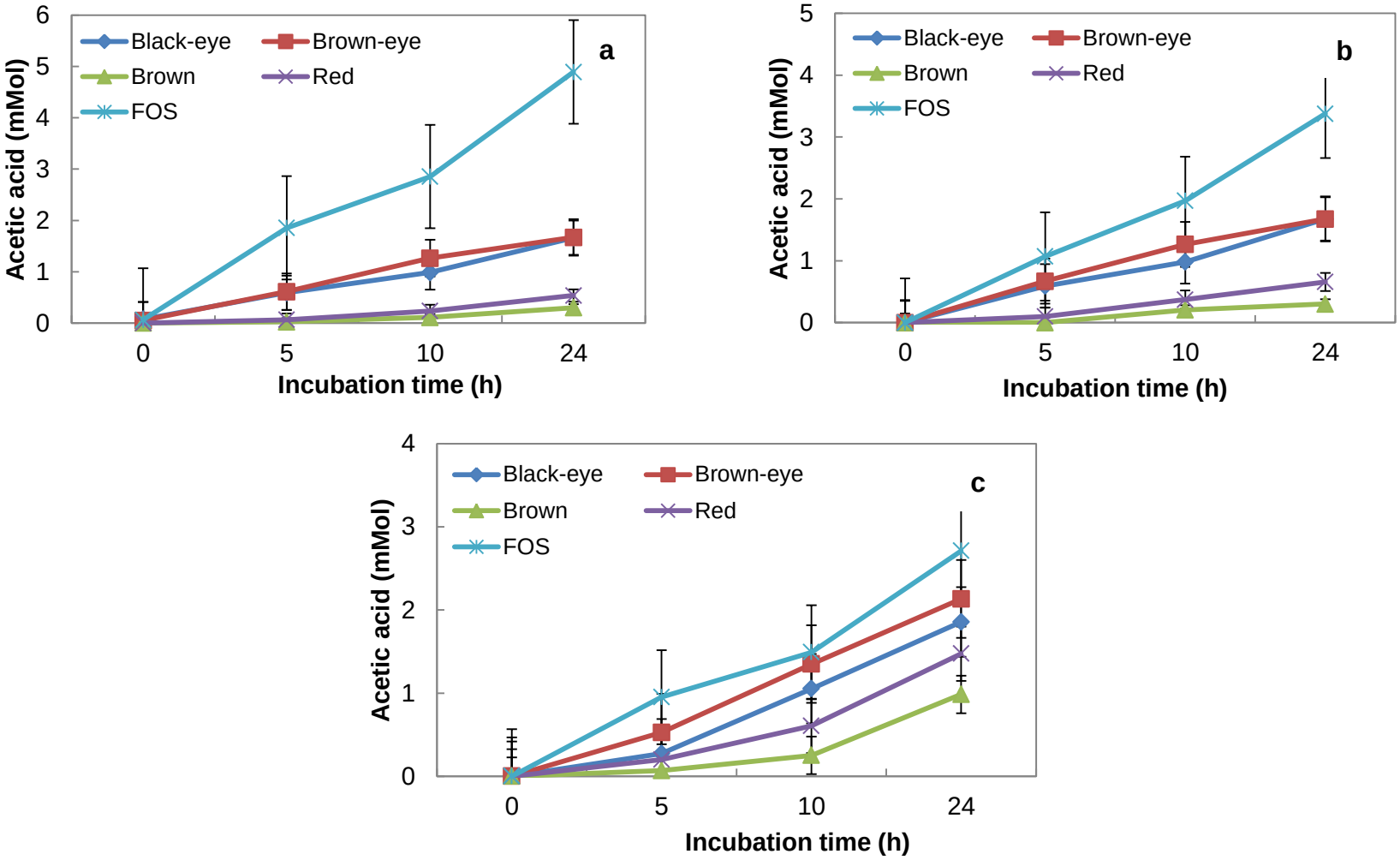

Figure 4.5 Acetic acid production after 24 h batch fermentation of Bambara groundnut soluble fibres and fructooligosaccharides by *Bifidobacterium* spp. (a) *B. breve* (b) *B. bifidum* (c) *B. animalis* subsp. *animalis.*

Acetic acid production by *B. bifidum* after 24 h was highest from the fermentation of black-eye and brown-eye SDFs (1.68 mMol) and lowest from the fermentation of brown SDF (0.30 mMol). Acetic acid produced from BGN SDF fermentation by *B. bifidum* was significantly different ($p < 0.05$). Under similar conditions, *B. bifidum* fermented millet SDF produced lower acetic acid quantities in the range 0.61 mMol to 0.68 mMol (Farroq *et al.*, 2013). These values relate to acetic acid produced in the fermentation of BGN red SDF (0.66 mMol) suggesting that red SDF could be a suitable substitute for millet SDF where prebiotic properties are desired. Fermentation of BGN SDFs by *B. animalis* subsp. *animalis* produced propionic acid in the range 0.86 mMol (brown SDF) to 1.83 mMol (black-eye SDF) after 24 h (Figure 4.4c).

FOS fermentation by *B*. *animalis* subsp. *animalis* yielded a significantly higher ($p < 0.05$) amount of propionic acid (4.79 mMol). After 24 h, the production of acetic acid from the fermentation of BGN SDFs by *B. animalis* subsp. *animalis* was in the range 2.71 mMol (brown-eye SDF) to 0.99 mMol (brown SDF) (Figure 4.5c). The fermentation of all BGN SDFs by *B. animalis* subsp. *animalis* yielded significantly ($p < 0.05$) different amounts of both propionic and acetic acids.

The production of SCFAs by *Bifidobacterium* spp. differed significantly ($p < 0.05$). The amounts of SCFAs produced in the absence of BGN SDFs and FOS (controls) were below limit of quantification (LOQ) indicating that SCFA production was mainly a result of BGN SDF fermentation. Propionic acid was produced in lesser quantities than acetic acid by all microorganisms which were in agreement with the reports of several researchers (Henningsson *et al.*, 2001; Vlkova *et al.*, 2002; Martin-Pelaez *et al*., 2008; Gietl *et al.*, 2012). BGN SDFs were degraded slower than FOS as was expected because longer carbohydrates are fermented at a slower rate (Toppin & Clifton, 2001; Rossi *et al*., 2005; Fuentes-Zaragoza *et al.*, 2011). In addition, FOS is highly prebiotic compared to many substrates (Probert *et al.*, 2004; Slavin, 2013). The differences in the SCFA quantities observed in this study and that of other studies can be attributed to the use of different substrates and different *Bifidobacterium* species (Henningsson *et al.*, 2001; Gietl *et al.*, 2012).

The production of these SCFAs is of importance in the maintenance of the integrity of the gut, immune system regulation, absorption of calcium and magnesium as well as regulation of blood cholesterol (Henningsson *et al*., 2001; Pan *et al.*, 2009; Fuentes-Zaragoza *et al.*, 2011). The genomic sequence of *Bifidobacterium* allows them to produce a number of diverse hydrolytic enzymes that degrade oligo- and polysaccharides to low molecular weight oligosaccharides which are subsequently broken down to monosaccharides (Rossi & Amaretti, 2010; Pokusaeva *et al.*, 2011). These monosaccharides are converted to intermediates of the hexose fermentation pathways also referred to as the bifid pathway using fructose-6-phosphate phosphoketolase ultimately producing SCFAs such as acetic acid and propionic acid (Tannock, 2010). Most constituents of dietary fibre are fermented by

anaerobic bacteria in the large intestine, producing SCFAs (Topping & Clifton, 2001; Andoh *et al.*, 2003; Wong *et al.*, 2006; Khan *et al*., 2007). The production of propionic acid from the fermentation of BGN SDFs was worth noting because *Bifidobacterium* spp. was previously reported to be poor producers of propionic acid in many substrates (Henningsson *et al*., 2001). Hence, the production of propionic acid from BGN SDF fermentation could be harnessed in the production of functional foods with particular emphasis on infant foods. *Bifidobacterium* spp. form a large part of the population of intestinal flora in infants (Amin *et al.*, 2013) therefore, BGN SDFs will play a major role in the proliferation of *Bifidobacterium*.

4.4 Conclusions

All four BGN SDFs were prebiotic in nature. Black-eye and brown-eye SDFs were the preferred substrates for the proliferation of *Bifidobacterium* spp. It can therefore be concluded that the inclusion of BGN SDFs in food systems would impart prebiotic properties to that particular system and hence increase its market value. It can further be concluded that BGN SDFs would be fermented by the microbiota in the human colon to produce SCFAs. These SCFAs are of importance to the physiological well-being of humans. The ability of BGN SDFs to promote the growth of *Bifidobacterium* spp. is an indication that these fibres can carry the "prebiotic" claim and can therefore be included in functional foods. *Bifidobacterium* spp. are generally abundant in the colon of breast feeding babies as well as in infants. BGN SDFs can thus be included in baby foods, especially for non-breast fed children, as alternatives to human oligosaccharides that breast fed children obtain from breast milk. BGN SDFs can be marketed as neutraceuticals.

References

Agarwal, N., Kamra, D.N., Chatterjee, P.N., Kumar, R. & Chaudhary, L.C. (2008). *In vitro* Methanogenesis, Microbial Profile and Fermentation of Green Forages with Buffalo Rumen Liquor as Influenced by 2-Bromoethanesulphonic Acid. Asian-*Australasian Journal of Animal Sciences*, **21**(6), 818 - 823.

Amin, M., Sheikh, A.F., Goodarzi, H. & Sormeh, M. (2013). Identification of *Bifidobacterium animalis, Bifidobacterium adolescentis* and *Bifidobacterium bifidum* from stool of children and detection of their antibacterial properties. *Advances in Infectious Diseases,* **3**, 200 – 204.

Andoh, A., Tsujikawa, T. & Fujiyama, Y. (2003). Role of dietary fibre and short chain fatty acids in the colon. *Current Pharmaceutical Design,* **9**(4), 347 - 358.

Asakuma, S., Hatakeyama, E., Urashima, T., Yoshida, E., Katayama, T., Yamamoto, K., Kumagai, H., Ashida, H., Hirose, J. & Kitaoka, M. (2011). Physiology of consumption

of human milk oligosaccharides by infant gut-associated Bifidobacteria. *The Journal of Biological Chemistry,* **286**, 34583 - 34592.

Baffoni, L., Stenico, V., Strahsburger, E., Gaggia, F., Gioia, D.D., Modesto, M., Mattarelli, P. & Biavati, B. (2013). Identification of species belonging to the *Bifidobacterium* genus by PCR-RFLP analysis of a *hsp60* gene fragment. *BMC Microbiology*, **13**, 149.

Barascu, E., Iordan, M. & Cimac, J. (2007). Growth rate of Bifidobacteria in milk supplemented with yeast extract and wheat germs. *The Annals of Valahia University - Targoviste,* **8**, 42 - 46.

Brock, T.D., Madigan, M.T., Martinko, J.M. & Parker, J. (1994). Growth and its control. In: *Biology of Microorganisms*. 7th ed. Pp. 324 – 325. New Jersey: Prentince-Hall International, Inc.

Collado, M.C. & Hernandez, M. (2007). Identification and differentiation of *Lactobacillus, Streptococcus* and *Bifidobacterium* species in fermented milk products with bifidobacteria. *Microbiological Research,* **162**(1), 86 - 92.

Collins, M.D. & Gibson, G.R. (1999). Probiotics, prebiotics, and synbiotics: Approaches for modulating the microbial ecology of the gut. *American Journal of Clinical Nutrition*, **69,** 1052 - 1057.

Corbo, M.R., Nobile, M.A.D. & Sinigaglia, M. (2006). A novel approach for calculating shelf life of minimally processed vegetables. *International Journal of Microbiology,* **106**, 69 – 73.

Cummings, J.H., Macfarlane, G.T. & Englyst, H.N. (2001). Prebiotic digestion and fermentation. *American Journal of Clinical Nutrition*, **73**, 415 - 420.

FAO. (2001). Food and Agriculture Organization of the United Nations. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Pp 20.

Farooq, U., Mohsin, M., Liu, X. & Zhang, H. (2013). Enhancement of short chain fatty acid production from millet fibres by pure cultures of probiotic fermentation. *Tropical Journal of Pharmaceutical Research,* **12**(2), 189 - 194.

Fernando, W.M.A.D.B., Flint, S., Zou, M., Brennan, C.S., Ranaweera, K.K.D.S. & Bamunuarachchi, A. (2011). The effect of rice fibre fractions on the growth of co-cultures of probiotics. *Journal of Food Science and Technology,* **48**(1), 14 - 25.

Fuentes-Zaragoza, E., Sanchez-Zapata, E., Sendra, E., Sayas, E., Navarro, C., Fernandez-Lopez, J. & Perez-Alvarez, J.A. (2011). Resistant starch as a prebiotic: A review. *Starch,* **63**, 406 - 415.

Gibson, G.R., Probert, H.M., Loo, J.V., Rastall, R.A. & Roberfroid, M.B. (2004). Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. *Nutrition Research Reviews*, **17**, 259 - 275.

Gietl, E., Mengerink, W., de Slegte, Gibson, G., Rastall, R. & van den Heuvel, E. (2012). Factors involved in the *in Vitro* fermentation of short carbohydrates in static faecal batch cultures. *International Journal of Carbohydrate Chemistry*, **2012**, 1 - 10.

Goderska, K., Nowak, J. & Czarnecki, Z. (2008). Comparison of the growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* species in media supplemented with selected saccharides including prebiotics. *ACTA Scientiarum Polonorum Technologia Alimentaria,* **7**(2), 5 - 20.

Guerzoni, M.E., Gianotti, A., Corbo, M.R. & Sinigaglia, M. (1996). Shelf-life modelling for fresh-cut vegetables. *Postharvest Biology and Technology,* **9**, 195 – 207.

Hessingsson, A., Bjorck, I. & Nyman, M. (2001). Short chain fatty acid formation at fermentation of indigestible carbohydrates. *Scandanavian Journal of Nutrition,* **45**, 165 - 168.

Hobden, M.R., Martin-Morales, A., Guérin-Deremaux, L., Wils, D., & Costabile, A. (2013). *In Vitro* Fermentation of NUTRIOSE® FB06, a Wheat Dextrin Soluble Fibre, in a Continuous Culture Human Colonic Model System. *PLoS ONE,* **8(**10), e77128.

Hur, S.J., Lim, B.O., Decker, E.A. & McClements, D.J. (2011). In vitro digestion models for food applicants. *Food Chemistry*, **125**, 1 - 12.

IBM Corp. (2013). IBM Statistical Package for the Social Science (SPSS), Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Janda, J.M. & Abbott, S.L. (2002). Bacterial Identification for Publication: When is enough enough?. *Journal of Clinical Microbiology,* **40**(6), 1887 - 1891.

Kaplan, H. & Hutkins, R.W. (2000). Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. *Applied Environmental Microbiology,* **66**(6), 2682 - 2684.

Khan, A.R., Alam, S., Ali, S., Bibi, S. Khalil, I.A. (2007). Dietary fibre profile of food legumes. *Sarhad Journal of Agriculture*, **23**(3), 763 - 766.

Kneifel, W., Rajal, A. 7 Kulbe, K.D. (2000). *In vitro* growth behaviour of probiotic bacteria in culture media with carbohydrates of prebiotic importance. *Microbial Ecology in Health and Disease,* **12**, 27 - 36.

Korakli, M., Ganzle, M.G. & Vogel, R.F. (2002). Metabolism by bifidobacteria and lactic acid bacteria of polysaccharides from wheat and rye, and exopolysaccharides produced by *Lactobacillus sanfranciscensis. Journal of Applied Microbiology,* **92**, 958 - 965.

Laird, D.T., Gambrel-Lenarz, S.A., Scher, F.M., Graham, T.E., Reddy, R & Maturin, L.J. (2004). Microbiological count methods. In: *Standard methods for the examination of dairy products*. Pp. 153. 17th ed. (Eds: H. M. Wehr & J.F. Frank). Washington: American Public Health Association

Lee, E.H.L., Degener, J.E., Welling, G.W. & Velo, A.C.M. (2011). Evaluation of the Vitek 2 ANC Card for Identification of Clinical Isolates of Anaerobic Bacteria. *Journal of Clinical Microbiology,* **49**(5), 1745 - 1749.

Lu, Z.X., Lu, F.X., Zhang, L.K., Bie, X.M. & Zou, X.K. (2006). Predictive modelling and growth models of aerobic mesophilic bacteria on fresh-cut lettuce by hypochlorite washing. *Journal of Food Safety,* **27**, 157 – 168.

Mahlen, S.D. & Clarridge III, J.E. (2009). Site and clinical significance of *Alloscardovia omnicolens* and *Bifidobacterium* species isolated in clinical laboratory. *Journal of Clinical Microbiology,* **53**(3), 3289 - 3293.

Manderson, K., Pinart, M., Tuohy, K.M., Grace, W.E., Hotchkiss, A.T., Widmer, W., Yadhav, M.P., Gibson, G.R. & Rastall, R.A. (2005). In vitro determination of prebiotic properties of oligosaccharides derived from an orange juice manufacturing by-product stream. *Applied and Environmental Microbiology*, **71**(12), 8383 - 8389.

Martin-Pelaez, S., Gibson, G.R., Martin-Orue, S.M., Klinder, A., Rastall, R.A., La Ragione, R.M., Woodward, M.J. & Costabile, A. (2008). *In vitro* fermentation of carbohydrates by porcine faecal inocula and their influence on *Salmonella* Typhimurium growth in batch culture systems. *Federation of European Microbiological Societies Microbial Ecology,* **66**, 608 - 619.

Moriya, J., Fachin, L., Gandara, A.L.N. & Viotto, W.H. (2006). Evaluation of culture media for counts of *bifidobacterium animalis* in the Presence of yoghurt bacteria. *Brazilian Journal of Microbiology*, **37**, 516 - 520.

Moore, K.E. (2011). Biological analysis of prebiotics in various processed food matrices. MSc in Food Science and Technology Thesis, University of Nebraska - Lincoln.

Palframan, R.J., Gibson, G.R. & Rastall, R.A. (2003). Carbohydrate preferences of *Bifidobacterium* species isolated from the human gut. *Current Issues in Intestinal Microbiology*, **4**, 71 – 75.

Pan, X.D., Chen, F.Q., Wu, T.X., Tang, H.G. & Zhao, Z.Y. (2009). Prebiotic oligosaccharides change the concentration of short chain fatty acids and the microbial population of mouse bowel. *Journal of Zhejiang University Science B,* **10**(4), 258 - 263.

Pokusaeva, K., Fitzgerald, G.F. & van Sinderel, D. (2011). Carbohydrate metabolism in Bifidobacteria. *Genes & Nutrition,* **6**(3), 285 - 306.

Probert, H.M., Juha H. A. Apajalahti, J.H.A., Rautonen, N., Stowell, J. & Gibson, G.R. (2004). Polydextrose, Lactitol, and Fructo-Oligosaccharide Fermentation by Colonic Bacteria in a Three-Stage Continuous Culture System. *Applied Environmental Microbiology*, **70**(8), 4505 - 4551.

Roberfroid, M. (2007). Prebiotics: The concept revisited. *Journal of Nutrition*, **137**, 830 - 837.

Rockova, S., Rada, V., Havlik, J., Svejstil, R., Vlkova, E., Bunesova, V., Janda, K. & Profousova, I. (2013). Growth of bifidobacteria in mammalian milk. *Czech Journal of Animal Science,* **58**(3), 99 - 105.

Rolfe, M.D., Rice, C.J., Lucchini, S., Pin, C., Thompson, A., Cameron, A.D.S., Alston, M., Stringer, M.F., Betts, R.P., Baranyi, J., Peck. M.W. & Hinton, J.C.D. (2012). Lag

phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. *Journal of Bacteriology,* **194**(3), 686 - 701.

Rossi, M. & Amaretti, A. (2010). Probiotic properties of Bifidobacteria. In: *Bifidobacteria: Genomics and molecular aspects.* (Eds: B. Mayo. & D. van Sinderel). Pp. 6. Norfolk: Caister Academic Press.

Rossi, M., Corradini, C., Amaretti, A., Nicolini, M., Pompei, A., Zanoni, S. & Matteuzzi, D. (2005). Fermentation of fructooligasaccharides and inulin by *Bifidobacteria*: A comparative study of pure and feacal cultures. *Applied and Environmental Microbiology,* **71**(10), 6150 - 6158.

Rycroft, C.E., Jones, M.R.M., Gibson, G.R. & Rastall, R.A. (2001). A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *Journal of Applied Microbiology,* **91**, 878 - 887.

Siedlecka, E.M., Kumirska, J., Ossowski, T.,Glamowski, P., Golebiowski, M., Gajdus, J., Kaczynski. & Stepnowski, P. (2008). Determination of volatile fatty acids in environmental aqueous samples. *Polish Journal of Environmental Studies,* **17**(3), 351 - 356.

Sinigaglia, M., Corbo, M.R., D'Amato, D., campaniello, D. & Altieri, C. (2003). Shelf-life modelling of ready-to-eat coconut. *International Journal of Food Science and Technology,* **38**, 547 – 552.

Slacanac, V., Lucan, M., Hardi, J., Habschied, K., Krstanovic. & Gagu;a, G. (2013). Predictive modelling of *Bifidobacterium animalis* subsp. *Lactis* Bb-12 growth in cow's, goat's and soy milk. *Mljekarstvo*, **63**(4), 220 – 227.

Slavin, J. (2013). Fibre and Prebiotics: Mechanisms and Health Benefits. *Nutrients,* **5**(4), 1417 - 1435.

Suharti, S., Astuti, D.A., Wina, E. & Toharmat, T. (2011).Rumen Microbial Population in the *In vitro* Fermentation of Different Ratios of Forage and Concentrate in the Presence of Whole Lerak (*Sapindus rarak*) Fruit Extract. *Asian-Australasian Journal of Animal Sciences*, **24**(8), 1086 - 1091.

Sutton, S. (2011). Measurement of microbial cells by optical density. *Journal of Validation Technology*, **2011**, 46 – 49.

Tannock, G. W. (2010). Analysis of Bifidobacterial populations in bowel ecology studies. In: *Bifidobacteria: Genomics and molecular aspects.* (Eds: B. Mayo. & D. van Sinderel). Pp. 1. Norfolk: Caister Academic Press.

Trojanova, I., Vlkova, E.., Rada, V. & Marounek, M. (2006). Different utilisation of glucose and raffinose in *Bifidobacterium breve* and *Bifidobacterium animalis. Folia Microbiology*, **51**(4), 320 - 324.

Todar, K. (2012). The growth of bacterial populations. In: Todar's online textbook of bacteriology. Pp. 2.

Topping, D.L. & Clifton, P.M. (2001). Short chain fatty acids and human colonic finction: Roles of resistant starch and non-starch polysaccharides. *Physiological Reviews*, **81**(3), 1031 - 1064.

Turroni, F., Foroni, E., Serafini, F., Viappiani, A., Montanini, B., Bottacini, F., Ferrarini, A., Baccchini, P.L., Rota, c., Delledonne, M., Ottonello, S., van Sinderen, D. & Ventura, M. (2011). Ability of *Bifidobacterium breve* to grow on different types of milk: Exploring the metabolism of milk through genome analysis. *Applied and Environmental Microbiology*, **77**(20), 7408 - 7417.

Turroni, F., Peano, C., Pass, D.A., Foroni, E., Severrgnini, M., Claesson, M.J., Kerr, C., Hourihane, J., Murray, D., Fuligni, F., Gueimonde, M., Margolles, A., De Bellis, G., O'Toole, P.W., van Sinderen, D., Marchesi, J.R. & Ventura, M. (2012). Diversity of Bifidobacteria within the infant gut microbiota. *PLoS One*, **7**(5), e36957.

Vigsnæs, L.K., Holck, J., Meyer, A.S. & Licht, T.R. (2011). *In Vitro* fermentation of sugar beet Arabino-Oligosaccharides by fecal microbiota obtained from patients with ulcerative colitis to selectively stimulate the growth of *Bifidobacterium* spp and *Lactobacillus* spp. *Applied and Environmental Microbiology*, **77**(23), 8336 - 8344.

Vlkova, E., Medkova, J. & Rada, V. (2002). Comparison of four methods for identification of *Bifidobacteria* to a genus level. *Czech Journal of Food Science,* **20***,* 171 - 174.

Vlkova, E., Nevoral, J., Jencikova, B., Kopecny, J., Godefrooij, J., Trojanova, I. & Rada, V. (2005). Detection of infant faecal bifidobacteria bt enzymatic methods. *Journal of Microbiological Methods,* **60**(3), 365 – 373.

Wong, J.M., de Souza, R., Kendall, C.W., Emam, A. & Jenkins, D.J. (2006). Colonic health: Fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology,* **40**(3), 235 - 243.

Ye, K., Wang, H., Zhang, X., Jiang, Y., Xu, X. & Zhou, G. (2013). Development and validation of a molecular predictive model to describe the growth of *Listeria monocytogenes* in vacuum-packaged chilled pork. *Food Control*, **32**, 245 – 254.

Zwietering, M.H, Jongenburger, F.M., Roumbouts, M. & van't Riet, K. (1990). Modelling of the bacterial curve. *Applied and Environmental Microbiology,* **56**, 1875 -1881.

CHAPTER FIVE

STABILITY AND RHEOLOGICAL PROPERTIES OF BAMBARA GROUNDNUT SOLUBLE DIETARY FIBRES ON ORANGE BEVERAGE EMULSION

Abstract

Bambara groundnut (BGN) soluble dietary fibres (SDF) from black-eye, brown-eye, brown and red BGN varieties were extracted and used to stabilise orange oil beverage emulsions at 6% orange oil and 30% SDF. Emulsion microstructures, droplet sizes and droplet size distributions were evaluated using image analysis. Emulsion stability was studied by observing changes in backscattering (BS) flux (%) at 20°C using a vertical analyser, Turbiscan MA 2000 as well as monitoring turbidity loss. Rheological properties assessed the time-dependent, steady shear and viscoelastic behaviour of emulsions using a shear controlled Rheometer. Brown-eye SDF and black-eye SDF stabilised emulsions showed similar microstructures. Black-eye SDF stabilised emulsion was characterised by the highest droplet volume (86%) and smallest droplet size (up to 3 µm). The volume-surface mean diameter ($d_{3,2}$) of the four emulsions ranged from 2.68 µm (brown SDF stabilised emulsion) to 17.09 µm (black-eye SDF stabilised emulsion) while the equivalent volume-mean diameter ($d_{4,3}$) ranged from 4.38 µm (brown SDF stabilised emulsion) to 18.62 µm (black-eye SDF stabilised emulsion). Emulsions were relatively stable to creaming and destabilised mainly by phenomenon involving oil droplet aggregation, however, differences in compositions of the dietary fibres significantly affected the flocculation kinetics of the emulsions. The BS percentage of the four emulsions was in the range 72.9% (brown SDF stabilised emulsion) to 85.0% (black-eye SDF stabilised emulsion). Black-eye SDF stabilised emulsion had the least turbidity loss rate (k) (0.070/day) and brown SDF stabilised emulsion had the highest turbidity loss rate (k) (0.221/day). All the emulsions were shear thinning (flow behaviour index <1), thixotropic and viscoelastic fluids. The emulsions stabilised with black-eye SDF showed the least hysteresis loop area (9.31 Pas^{-1}), highest apparent viscosity (1.6 Pas^{-1}) and exhibited storage modulus (G') positioned above loss modulus (G''). All the SDFs from the four BGN varieties greatly indicated their potential in stabilising beverage emulsions. Principal component analysis (PCA) was used to reduce the variability of data among the four SDF stabilised emulsions. Component 1 accounted for 79.1% of variability and component 2 accounted for 17.2% of variability. Component 1 was shown to represent volume-surface mean diameter ($d_{3,2}$) (0.997), equivalent volume-mean diameter ($d_{4,3}$) (0.994), hysteresis loop area (0.983) as well as the consistency coefficient (K) (0.215). Component 2, showed strong association with consistency coefficient (K) (0.976), backscattering (0.205) and a lesser association with volume-surface mean diameter ($d_{3,2}$) (0.009).

5.1 Introduction

Beverage emulsions are defined as oil-in-water emulsions that can be classified as flavour emulsions or as cloud emulsions (Reiner *et al*., 2010, Gharibzahedi *et al.*, 2012; Cheong *et al*., 2014). In beverages such as fruit drinks, sodas and punches, cloud emulsions provide opaqueness and flavour emulsions provide flavour in addition to cloudiness (Harnsilawat *et al.*, 2006; Mirhosseini *et al*., 2008). In these emulsions, the oil phase is the flavour oil such as terpenes or vegetable oil and the water phase is a solution of highly functional hydrocolloids (Gharibzahedi *et al.*, 2012). The instability of emulsions is a challenge especially to beverage manufacturers as beverages are very dilute with as little as 20 mg/L oil phase present in the final product (Reiner *et al*., 2010; Rezvani *et al.*, 2011). Beverages are expected to be stable, both as concentrates and as dilute, for a period of at least 6 months according to beverage standards (Yadav, 2007; Mirhosseini *et al*., 2008).

The instability of beverage emulsions can be attributed to the positive free energy present during the formation of dispersions (Gharibzahedi *et al*., 2012). This makes the whole system prone to separation through creaming, coalescence, flocculation and sedimentation (Mengual *et al.*, 1999; Chanamai & McClements, 2000). Furthermore, it must be taken into consideration that some constituents such as colourings, flavourings and other additives are not entirely dissolved making the system inherently unstable and thus at risk of dissociating (Desplanque *et al*., 2012). To increase the kinetic stability of beverages, approaches such as homogenization and the inclusion of stabilisers, weighting agents, emulsifiers including synthetic surfactants, phospholipids and polysaccharides are adopted (Chanamai & McClements, 2000; Rezvani *et al.*, 2011; Cheong *et al*., 2014).

Orange terpenes or medium chain triglycerides are usually used by the flavour industry in preparation of cloud emulsions (Harnsilawat *et al.*, 2006; Reiner *et al*., 2010). Phytochemicals found in orange oil may contribute health promoting effects such as anti-carcinogenic and anti-inflammatory activities (Rezvani *et al.*, 2011). The low densities of essential oils however render them unstable if poorly controlled (Taherian *et al.*, 2010). Hydrocolloids such as xanthan gum, galactomanans, starches, gum Arabic and polysaccharides play a major role in stabilising beverage emulsions (Acton, 2012; Gharibzahedi *et al.*, 2012). These have texturing properties that help in the controlling of rheological properties and stability of oil in water emulsions (Rezvani *et al.*, 2011; Desplanque *et al*., 2012). In order to produce a stable beverage emulsion, it is of utmost importance that the hydrocolloid adsorbs during emulsification so as to create the necessary stabilising layer and therefore a stable emulsion (Gharibzahedi *et al.*, 2012).

Various tests and instrumentation for characterising the physicochemical characteristics of emulsions have been developed over the years. These give data on the change in droplet size and concentration with time (Gabriel *et al.*, 2013). The Turbiscan was employed in this study. This instrument is based on the principle of light scattering in which a

monochromatic beam of near infrared light is directed through an emulsion placed in a vertical flat-bottomed glass tubes. Hence, the amount of transmitted or backscattered light is measured as a function of the height of the emulsion (Blijdenstein *et al*., 2004). This can be used to determine creaming and/or sedimentation as information on the changes in droplet concentrations along the height of the emulsion is obtained. The Turbiscan is fitted with two detectors namely the transmission detector and backscattering detector (Mengual *et al*., 1999; Diedericks, 2014). The former receives light passing through the sample and the latter receives light reflected by the sample. Flocculation is observed in the optimum zone of 20 – 50 mm and creaming is observed as a peak in delta backscattering curves between 0 – 20 mm (Alvarez Cerimedo *et al*., 2010). Optical microscopy is also commonly employed to determine growth of emulsion droplet which is an indication of emulsion instability (Gabriel *et al.*, 2013). From the microscopic images, information about droplet sizes and droplet size distribution can be obtained (Adeyi, 2014).

Gum Arabic is the most popularly used stabiliser in beverage emulsions but has limitations due to its high cost, lack of consistency in quality and lack of reliable sources (Yadav *et al.*, 2007; Gharibzahedi *et al.*, 2012; Cheong *et al*., 2014). As such there is a need for a suitable substitute and BGN SDF has potential as an alternative. Bambara groundnut has not gained the popularity it deserves although the potential of its components as emulsion stabilisers has been demonstrated. Gabriel *et al.* (2013) demonstrated the emulsifying properties of BGN starch, Adeyi (2014) reported the emulsion stabilising properties of BGN flour and Diedericks (2014) reported the emulsion stabilizing properties of BGN fibre from the brown variety. Dhingra *et al.* (2011) stated that fibre ingredients are useful in increasing emulsion stability and viscosity in beverages. BGN soluble dietary fibre (30%) and orange oil (6%) were used in this study as this combination was concluded to be the optimum that gave the most stable concentrated beverage emulsion by Diedericks (2014). However, nothing is reported about the stability of Bambara groundnut (BGN) soluble dietary fibre (SDF) stabilised emulsions when diluted. The aim of this study was to evaluate BGN soluble dietary fibres as potential orange oil beverage emulsion stabilisers with a view to provide the beverage industry with a low cost, alternative stabiliser.

5.2 Materials and Methods

5.2.1 Source of materials

Soluble dietary fibres (SDFs) from four varieties of BGN (black-eye, brown-eye, brown and red) were used in this study. The SDFs were extracted following the procedures detailed in section 3.2.3 (page 54). Cold pressed orange oil (Puris Natural Aroma Chemicals, South Africa) was used as the oil phase and deionised water was used as the water phase. Commercial grade citric acid, potassium sorbate and sodium benzoate (Sigma-Aldrich,

Johannesburg, South Africa) were used in the preparation of the beverage emulsion. Figure 5.1 outlines the different analyses that were carried out in this chapter.

5.2.2 Preparation of beverage emulsions

The method of Diedericks (2014) was adopted in this study. Emulsions were prepared using each of the four varieties of BGN SDFs (black-eye, brown-eye, brown and red). A formulation containing (w/w) 30% BGN SDF, 6% orange oil, 0.4% citric acid, 0.1% sodium benzoate, 0.1% potassium sorbate and deionised water was used in the preparation of the orange oil beverage emulsion. BGN (30%) and orange oil (6%) were used in this study as this combination was concluded to be the optimum concentration that gave the most stable emulsion by Diedericks (2014). Initially, the appropriate amounts of citric acid, sodium benzoate, potassium sorbate and deionised water (60°C) were mixed for 2 min at low speed (Waring blender). Bambara groundnut SDF was then added to the mixture slowly to avoid lumping and blended for another 2 min at high speed. Orange oil was then added to the mixture and blended at high speed for 2 min. The resultant emulsion was homogenised for 5 min at 12000 rpm (Ultra Turrax homogeniser, IKA T25 digital, Janke and Kunkel, Staufen, Germany). Immediately after homogenisation, the kinetic stability and microstructures of the emulsions stabilised with black-eye, brown-eye, brown and red BGN SDFs were analysed individually. Three replicates of each emulsion were prepared.

5.2.3 Emulsion microstructure analysis

The microstructure of each freshly prepared emulsion was assessed using a digital microscope (Ken-a-Vision TU-19542C, Ken-a-Vision Mfg Co. Inc., USA) mounted with a digital camera. Each emulsion was diluted with deionised water at a ratio 1:3 (w/w), [emulsion:water] to avoid overlapping of oil droplets which would make it difficult to visualise individual droplets. A single drop of each emulsion was placed on a microscope slide, covered with a cover slip and observed at 100X magnification. Images were recorded using Applied Vision 4 software (Ken-a-Vision Mfg Co. Inc., version 4.1.12, USA).

5.2.4 Quantification of droplet sizes and droplet size distribution of emulsions by image analysis

Droplet sizes and droplet size distributions of the four emulsions were assessed using images obtained from the prepared emulsions (Section 5.2.3). Image processing and further analysis were carried out using software Image J v1.36b. The diameters of the oil droplet were measured individually according to the method of Tcholakova *et al.* (2004). A number of droplets (n = 100) were counted in order to obtain a statistical estimate of the oil droplet diameters and oil droplet distribution for each sample. Droplet size distribution was generated by categorising the classes belonging to a common interval.

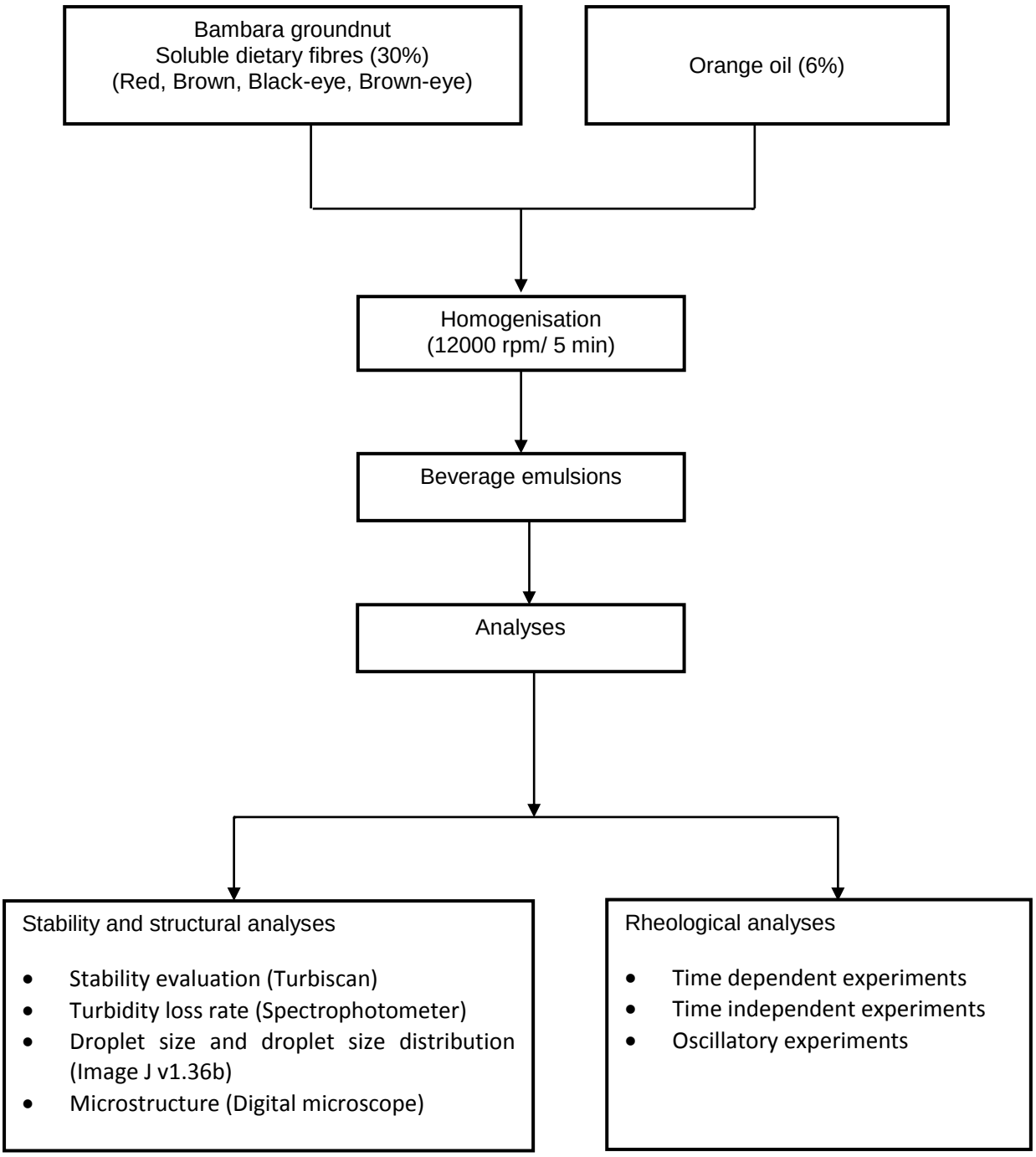

Figure 5.1 Experimental design for Chapter 5.

Droplet size frequency distribution was analysed using Microsoft™ Excel 2007. Oil droplet sizes were obtained in terms of volume-surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter ($d_{4,3}$) according to equations 3.7 and 3.8 respectively.

$$d_{3,2} = \frac{\sum n_i d_{i3}}{\sum n_i d_{i2}} \qquad \textit{Equation 3.7}$$

$$d_{4,3} = \frac{\sum n_i d_{i4}}{\sum n_i d_{i3}} \qquad \textit{Equation 3.8}$$

Where n_i is the number of droplets with diameter d_i.

5.2.5 Emulsion stability evaluation

The Turbiscan stability analysis (Turbiscan MA 2000, Formulaction, Toulouse, France) was carried out to determine the stability of the four BGN SDF stabilised beverage emulsions according to the method of Adeyi (2014). For each emulsion, a 7 mL sample in a Turbiscan tube (65 mm length) was scanned. The measurement involved scanning each sample along its height for 1 h at 10 min intervals. The backscattering (BS) and transmission curves generated were used to provide the BS and transmission flux percentage relative to the instrument's internal standard as a function of the height of the sample. Stability and instability of the emulsions were observed and analysed by carrying out multiple scans. Each scan provided a curve and all curves were laid on a single graph. From these scans, stability or instability was observed. Creaming is generally observed as a decrease and increase in BS flux at the bottom and top of the sample. Coalescence and flocculation though physiologically different, are deemed the same in Turbiscan analysis and are observed as a decrease in BS over the whole height of the sample (Alvarez-Cerimedo *et al.*, 2010).

5.2.6 Emulsion turbidity loss rate assessments

The turbidity of the four BGN SDF stabilised beverage emulsions were individually determined by diluting the beverage emulsions in a 10% sugar solution to 0.25% (w/w) and storing the diluted emulsions at room temperature (23 - 25°C) in plastic bottles. Absorbance readings were then taken using a temperature controlled (20°C) UV-visible spectrophotometer (UV-1700 PharmsSpec, Shimadzu, Japan) at a wavelength of 500 nm. Plastic cuvettes (Macro PS, Lasec, 10X10X45 mm) were used. Measurements were carried out as described by Gharibzahedi *et al.* (2012), and the turbidity loss rate determined as k by using the first-order model as shown in equation 5.1.

$$\ln A = \ln A_0 - kt \qquad \textit{Equation 5.1}$$

Where t is time, A is the absorbance at time *t*, A_0 is the absorbance at time 0 and *k* is the first-order rate constant.

5.2.7 Rheological measurements of emulsions stabilised with BGN SDFs

The rheological properties of the four emulsions stabilised with BGN SDFs were evaluated using an MCR 300 Paar Physical Rheometer (Discovery HR-1, hybrid rheometer). All measurements were conducted at 20°C. The methods of Adeyi (2014) were adopted to evaluate the shear-dependent/time independent, time dependent and viscoelastic properties of the emulsions.

1. *Time dependent rheological measurements (hysteresis loop)*

Time dependent rheological experiments of the emulsions were conducted without previous shearing. Emulsions (25 mL) were carefully transferred into the rheometer cup and allowed to equilibrate for 5 min. The change in viscosity was then measured as a function of increasing shear rate from 0.01 to 1000 s^{-1} followed by a decreasing shear rate from 1000 to 0.01 s^{-1}. In order to describe the time dependent flow behaviour, experimental data (shear stress-shear rate) of forward and backward curves were fitted to Power law model. The hysteresis loop area (Equation 3.9) was calculated as the area between the upstream data and downstream data (Tarrega *et al.,* 2004; Koocheki & Razavi, 2009). Each experiment was performed in duplicate.

$$\int_{\gamma_1}^{\gamma_2} K\gamma^{n} - \int_{\gamma_1}^{\gamma_2} K'\gamma^{n'} \qquad \textit{Equation 3.9}$$

Where, K, K' are the consistency coefficients and n, n' are the flow behavior indices for upward and downward measurements, respectively.

2. *Time independent rheological measurements (steady shear)*

The response of the viscosity of BGN SDF stabilised emulsions to variations in shear rate was evaluated following the method of Sahin & Sumnu (2006). To conduct steady state shear experiments, emulsions (25 mL) were carefully transferred into the rheometer cup and allowed to equilibrate for 5 min before shearing proceeded. Emulsion viscosity was measured over a shear rate range of 0.01 – 500 s^{-1} for a duration of 300 s. All measurements were performed in duplicate. Experimental flow data were evaluated and fitted according to the Power law rheological model (Equation 3.10).

$$\tau = K\gamma^{n} \qquad \textit{Equation 3.10}$$

Where: n = flow behaviour index which indicates the tendency of a fluid to shear thinning, *K* = consistency efficiency, γ = shear rate, τ = shear stress.

3. *Oscillatory experiments*

Each emulsion (25 mL) was carefully transferred into the rheometer cup and allowed to rest for 5 min before oscillatory analyses proceeded. Frequency sweep experiments were conducted at a constant frequency of 1 Hz and the storage and loss moduli were recorded as a function of stress (0.01 – 100 Pa). In order to determine the linear viscoelastic region, the storage modulus was plotted against shear stress. A shear stress of 0.1 Pa was afterwards selected for further frequency sweep experiments. Frequency sweep experiments were conducted over a frequency range of 0.628 – 62.8 rad/s at a constant stress of 0.1 Pa. The fingerprint of each emulsion in terms of storage and loss moduli was then plotted as a function of frequency. All experiments were conducted in duplicate.

5.2.8 Data analysis

IBM Statistical Package for the Social Science (IBM SPSS, version 22, 2013) was used. The results were subjected to Multivariate Analysis of Variance (MANOVA) to determine mean differences between treatments. Duncan's multiple range test was conducted to separate mean differences where differences exist. Principal component analysis (PCA) was used for data reduction. To describe rheological data so as to predict the behaviour of BGN SDF stabilised emulsions, Power law rheological model was used (Equation 3.10). The goodness-of-fit of the models was assessed using the coefficient of determination (R^2). Results were expressed as mean ± standard deviation and as mean ± standard error for the turbidity loss rate model parameters.

5.3 Results and Discussion

5.3.1 Effect of BGN SDFs on droplet size and droplet size distribution of orange oil beverage emulsion

The four emulsions stabilised with BGN are shown in Figure 5.2 and their droplet size distribution is shown in Figure 5.3. The droplet size distribution of the emulsion stabilised with black-eye SDF had the highest height and the smallest width. This indicated that black-eye SDF emulsion was characterised by the highest droplet volume (86%) and the smallest droplet size (up to 3 µm). The curves for brown and red SDF stabilised emulsions had the shortest heights (38% and 39% respectively) and both had broader bases than the black-eye

a b c d

Figure 5.2 Orange oil beverage emulsions stabilised with 30% Bambara groundnut soluble dietary fibres (a) Black-eye (b) Brown-eye (c) Brown (d) Red.

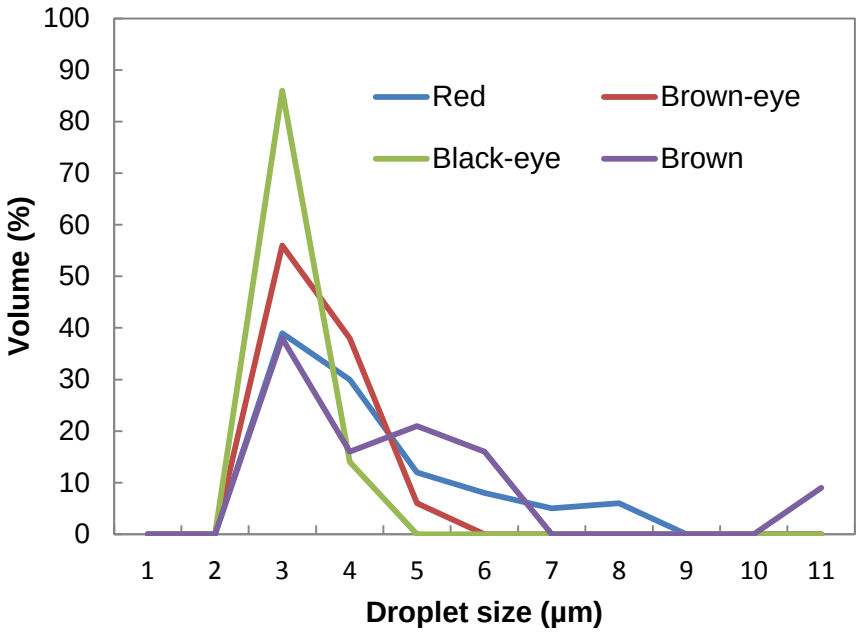

Figure 5.3 Droplet size and droplet size volume of Bambara groundnut soluble dietary fibre stabilised emulsions.

and brown-eye SDF stabilised emulsions curves (Figure 5.3). This indicated that these emulsions had larger oil droplets and the volume of the droplets was low. The droplet size distribution of brown and red SDF stabilised emulsions did not differ significantly ($p > 0.05$). Behrend *et al.* (2000) explained that the addition of stabilisers to an emulsion reduces its droplet size. However, the stabilising properties of the system determine the coalescence of droplets after disruption (homogenisation) thereby determining the final droplet size distribution. Therefore, black-eye SDF stabilised emulsion could be deduced to have the highest strength and matrix to stabilise emulsions among BGN SDFs.

Brown and red SDF stabilised emulsions could be assumed to be the least stable amongst the four emulsions because larger droplet sizes in emulsion systems have a greater tendency of coalescence having higher impact and magnitude during collision (Behrend *et al.*, 2000; Adeyi, 2014). None of the distribution (Figure 5.3) showed a perfect bell shape and all tended to have a shoulder which could be an indication of the presence of a second population (Chanamai *et al*., 2000). This could mean that all the emulsions were poly-dispersed in nature (Kumar *et al*., 2012; Adeyi, 2014). Hence, all four BGN SDFs were capable of forming emulsions with orange oil.

Table 5.1 gives a comparison of the droplet sizes of the four emulsions in terms of volume-surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter ($d_{4,3}$). The volume-surface mean diameter ($d_{3,2}$) of the four emulsions ranged between 2.68 and 17.09 µm while the equivalent volume-mean diameter ($d_{4,3}$) ranged between 4.38 and 18.62 µm. The smallest and highest volume-surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter ($d_{4,3}$) were found in emulsions stabilised with black-eye and brown SDFs, respectively. The volume-surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter ($d_{4,3}$) of all four BGN SDF stabilised emulsions were significantly ($p < 0.05$) different. Volume-surface mean diameter ($d_{3,2}$) is the volume-surface mean diameter of emulsions and gives information regarding size of emulsion where most droplets fall. Volume-mean diameter ($d_{4,3}$) is the equivalent volume-mean diameter and is a measure of changes in droplet size involving emulsion destabilisation process.

A relatively smaller volume-surface mean diameter ($d_{3,2}$) observed in black-eye SDF stabilised emulsion indicated the presence of small droplet sizes within the system while a relatively higher volume-surface mean diameter ($d_{3,2}$) observed in brown SDF stabilised emulsion indicated the presence of larger droplet sizes. These observations were in agreement with the results reported in the previous section (Figure 5.3) where black-eye SDF stabilised emulsion was shown to have the smallest droplet sizes while brown showed the largest droplet sizes. In all cases, the equivalent volume-mean diameter ($d_{4,3}$) was higher that volume-surface mean diameter ($d_{3,2}$) as expected (Adeyi, 2014). The stability of emulsions is dependent on equivalent volume-mean diameter ($d_{4,3}$) and volume-surface

Table 5.1 Effect of Bambara groundnut soluble dietary fibres on oil droplet size

Soluble fibre variety	$d_{3,2}$ (µm)	$d_{4,3}$ (µm)
Black-eye	2.68 ± 0.48^{a}	4.38 ± 0.25^{a}
Brown-eye	4.92 ± 0.59^{b}	5.72 ± 0.06^{b}
Red	9.82 ± 0.11^{c}	11.08 ± 0.07^{c}
Brown	17.09 ± 0.07^{d}	18.62 ± 0.11^{d}

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different. $d_{3,2}$: volume-surface mean diameter. $d_{4,3}$: equivalent volume-mean diameter.

mean diameter ($d_{3,2}$), with smaller values indicating more stable emulsions (Abismail *et al.*, 1999; Chanamai *et al,*. 2000; Mirhosseini *et al.*, 2008). Consequently, black-eye SDF stabilised emulsion was the most stable emulsion while brown SDF stabilised emulsion was the least stable. Since all the emulsions were prepared with the same concentration of BGN SDF and orange oil, the differences in droplet sizes and droplet size distribution could be attributed to varietal differences.

5.3.2 Effect of BGN SDFs on orange oil emulsion microstructure

The microstructures of freshly prepared emulsions stabilised with BGN SDFs are shown in Figure 5.4. The small droplets observed in the micrographs represent the dispersed phase which is the orange oil and the empty spaces represent the continuous phase which is the BGN SDF dispersion. The strand-like clumps are probably BGN SDF within the system.

Black-eye SDF stabilised emulsion (Figure 5.4a) showed very small oil droplets dispersed evenly throughout the system as well as smaller clumps of SDF. This is an indication that black-eye SDF had more emulsion forming strength. Brown-eye SDF stabilised emulsion (Figure 5.4b) showed a microstructure similar to that of black-eye stabilised emulsion. Brown and red SDF stabilised emulsions (Figures 5.4c and 5.4d) showed coalescence as small oil droplets were adsorbed on the surface of larger ones as well as flocculation as groups of oil droplets clumped together (Adeyi, 2014). The microstructures of brown and red SDF stabilised emulsions showed droplet aggregation which could be attributed to destabilisation by flocculation and creaming. Consequently, the oil droplets of the brown and red SDF stabilised emulsions would aggregate and thus destabilise at a faster rate than black-eye and brown-eye SDF stabilised emulsions over time thus making them less stable (Mirhosseini *et al.*, 2008).

Black-eye and brown-eye SDF stabilised emulsions micrographs indicated that black-eye and brown-eye SDFs had a higher strength for emulsion formation than red and brown SDFs. In Chapter 3, [section 3.4.4, page 72] black-eye and brown-eye SDFs were shown to have higher oil binding capacities of 3.84 and 4.03 g oil/g fibre, respectively while red and brown SDFs had lower oil binding capacities of 3.72 g oil/g fibre and 2.78 g oil/g fibre, respectively. Oil binding capacities (OBCs) of polysaccharides affect their emulsion stabilising properties with those having higher OBCs binding the oil phase more than those with lower OBCs (Adeyi, 2014). The OBCs of BGN SDFs could therefore explain the more dispersed emulsion systems stabilised with black-eye and brown-eye SDFs and the least dispersed emulsion systems stabilised with red and brown SDFs. Abismail *et al.* (1999) and Aveyard *et al.* (2002) reported that stabilisers cover the temporary interface preventing oil droplets from coalescing and therefore results in a lower volume-surface mean diameter ($d_{3,2}$).

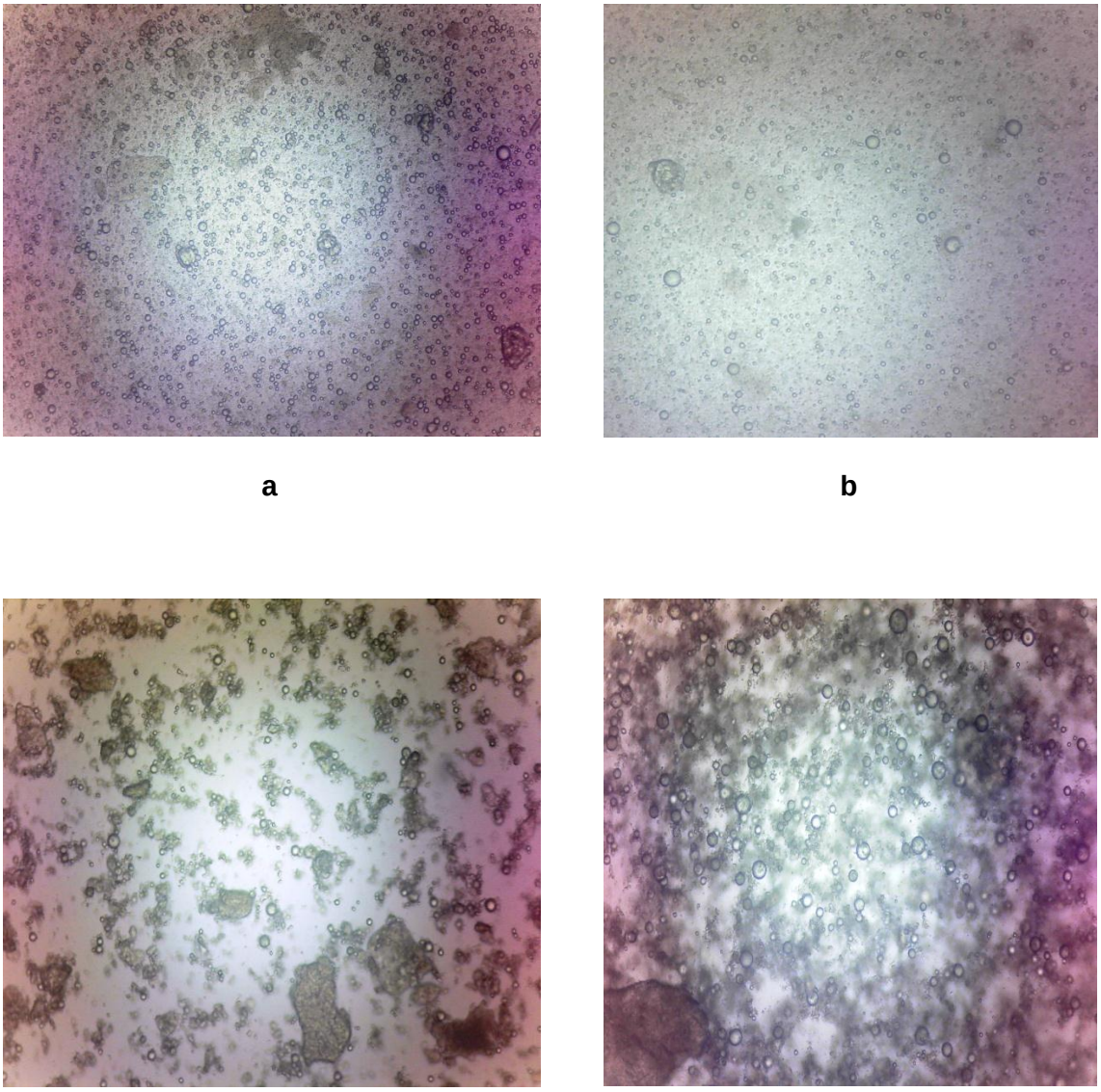

Figure 5.4 Photo micrographs of emulsion stabilised with Bambara groundnut soluble dietary fibres and orange oil (a) Black-eye (b) Brown-eye (c) Brown (d) Red.

From this study, it can be concluded that black-eye and brown-eye SDFs completely covered the temporary interface thereby gave rise to smaller droplets.

5.3.3 Storage stability of BGN fibre stabilised emulsions

Table 5.2 shows the effect of black-eye, brown-eye, brown and red BGN SDFs on the initial backscattering (BS) of emulsions. The BS flux percentage of the four SDF stabilised emulsions ranged from 72.9% (brown) to 85.0% (black-eye). All four emulsions differed significantly ($p < 0.05$) in respect to BS flux percentage. However, Diedericks (2014) reported lower BS values (62.99%) for BGN SDF stabilised emulsions (30% SDF/ 6% oil). Backscattering gives an indication of the structure of the emulsion before destabilisation thereby the highest percentage indicated initial high stability (Mengual *et al.*, 1999). A higher BS percentage indicates that the emulsion consists of a higher population of oil droplets with small droplet sizes that disperse a higher amount of light (Adeyi, 2014). From Table 5.2, black-eye SDF stabilised emulsion could be deduced to be the most stable system as it had the highest BS percentage meaning the emulsion had relatively smaller, evenly dispersed droplets (Adeyi, 2014; Diedericks, 2014).

Figure 5.5 shows the Turbiscan profiles of the four emulsions. The x-axis represents the height of the tube and the y-axis represents the BS flux percentage. The Turbiscan profiles (Figure 5.5) are represented by the normal mode (above) and their respective reference mode (below). The Turbiscan profiles of all four emulsions follow the same path as the initial scan. However the scans did not overlay perfectly showing a decrease in BS flux percentage. This observation in all four emulsions indicated that the main phenomenon of disintegration in the emulsions was particle aggregation which could be attributed to either flocculation or coalescence. This was in agreement with Diedericks (2014) who reported that BGN SDF stabilised emulsions destabilised by flocculation.

The reference modes of red and brown SDF stabilised emulsions (Figure 5.5c and 5.5d, respectively) showed more separation between the various scans compared to those of black-eye and brown-eye SDF stabilised emulsions (Figures 5.5a and 5.5b, respectively) indicating that red and brown SDF stabilised emulsions were relatively less stable and showed higher destabilisation due to droplet aggregation. These observations agreed with the microstructure and droplet size analyses [Sections 5.3.1 and 5.3.2, page 149,153] which revealed that larger oil droplets was associated with brown and red SDF stabilised emulsions and smaller, more evenly dispersed oil droplets associated with black-eye and brown-eye SDF stabilised emulsion. Reference modes were constructed relative to the normal scan and were used to show relative changes with time in all four emulsions. The reference modes allow better visualisation and analysis of the stability of emulsion systems. From the reference modes, the type of mechanism of disintegration of the emulsion could be observed.

Table 5.2 Effect of Bambara groundnut (BGN) soluble fibres on initial backscattering values of emulsions

BGN stabilised emulsion	Initial backscattering value (%)
Black-eye	85.0 ± 0.27^{a}
Brown-eye	82.7 ± 0.45^{b}
Red	75.7 ± 0.02^{c}
Brown	72.9 ± 0.08^{d}

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different.

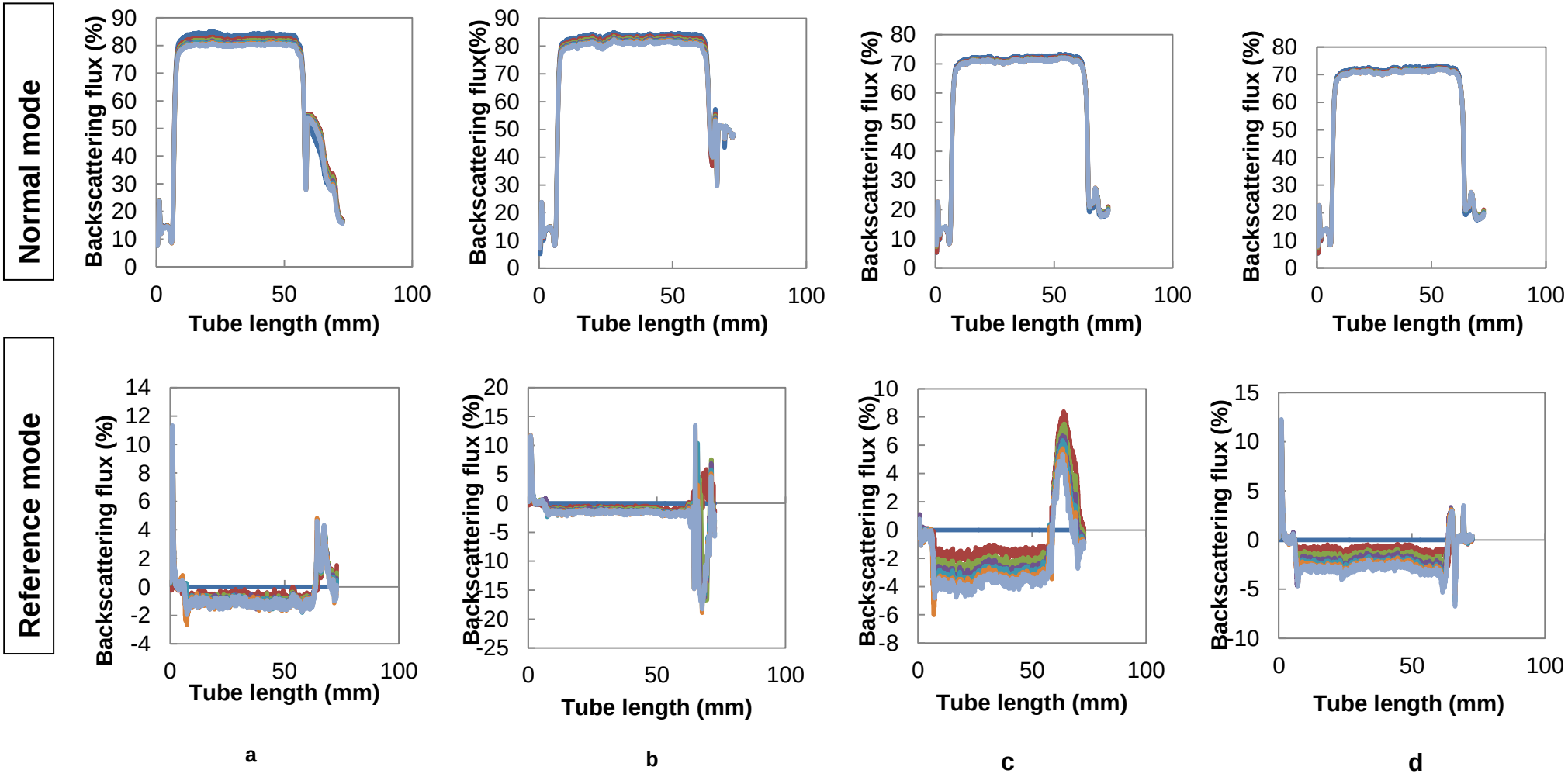

Figure 5.5 Changes in backscattering profile as a function of sample height during storage of emulsions stabilised by four varieties of Bambara groundnut soluble dietary fibres (a) Black-eye (b) Brown-eye (c) Brown (d) Red.

The normal modes of Turbiscan gave repeated scans over time and from those graphs, the initial BS percentage was calculated and thus information on the microstructure of the emulsions was obtained.

Figure 5.6 shows the effect of BGN SDFs on the BS flux (%) kinetics, indicating how they compared to each other in terms of stability over time. To quantify the changes in particle size variation such as coalescence and flocculation in the emulsions, the variation in backscattering in the 20 – 40 mm zone was monitored over 60 min for samples stored in inert condition at 20°C. The further the graphs were from the origin the less stable the emulsion was. Hence, graphs that were closer to zero at any given time were considered more stable. Black-eye SDF gave the most stable emulsion while brown SDF gave the least stable emulsion as shown in Figure 5.6. These results were in agreement with those given in the previous sections where it was established that black-eye SDF stabilised emulsion was the most stable emulsion characterised by smaller oil droplets and highest droplet volume while brown SDF stabilised emulsion was the least stable characterised by larger oil droplets as well as the lowest droplet volume.

Physical stability of emulsions is largely dependent on droplet size (Behrend *et al.*, 2000; Chanamai *et al.*, 2000; Mirhosseini *et al.*, 2008; Dickinson, 2008). BGN SDFs acted as stabilisers in the emulsions systems. Stabilisers are defined by Behrend *et al.* (2000) as non-surface active macromolecules which thicken the continuous phase thereby decreasing the mobility of oil droplets therefore preventing them from coalescing.

5.3.4 Turbidity loss rates of BGN soluble dietary fibre stabilised emulsions

Figure 5.7 shows images of the four diluted emulsions [1:400 (w/w)], emulsion:sugar solution. Black-eye and brown-eye SDF stabilised emulsions showed more cloudiness than red and brown SDF stabilised emulsions. Concentrated beverage emulsions are diluted into finished products, with the finished product having as little as 20 mg/L oil phase (Chanamai & McClemments, 2001; Reiner *et al*., 2010).

Figure 5.8 shows the plots of the logarithm (Ln) of absorbance as an indicator of turbidity loss rate for the four BGN SDF stabilised emulsions. All four BGN SDF stabilised emulsions showed a decrease in turbidity with storage as evidenced by the negative slopes on the graphs. Table 5.3 shows the constrained non-linear regression parameters of the turbidity loss rate model (Equation 5.1). The absorbance (A) of the BGN SDF stabilised emulsions ranged from 0.181 (brown SDF stabilised emulsion) to 0.25 (brown-eye SDF stabilised emulsion) and all differed significantly ($p < 0.05$). Black-eye SDF stabilised emulsion had the least ($p < 0.05$) turbidity loss rate (k) of 0.070/day and brown-eye SDF stabilised emulsion had the highest ($p < 0.05$) turbidity loss rate (k) (0.221/day).

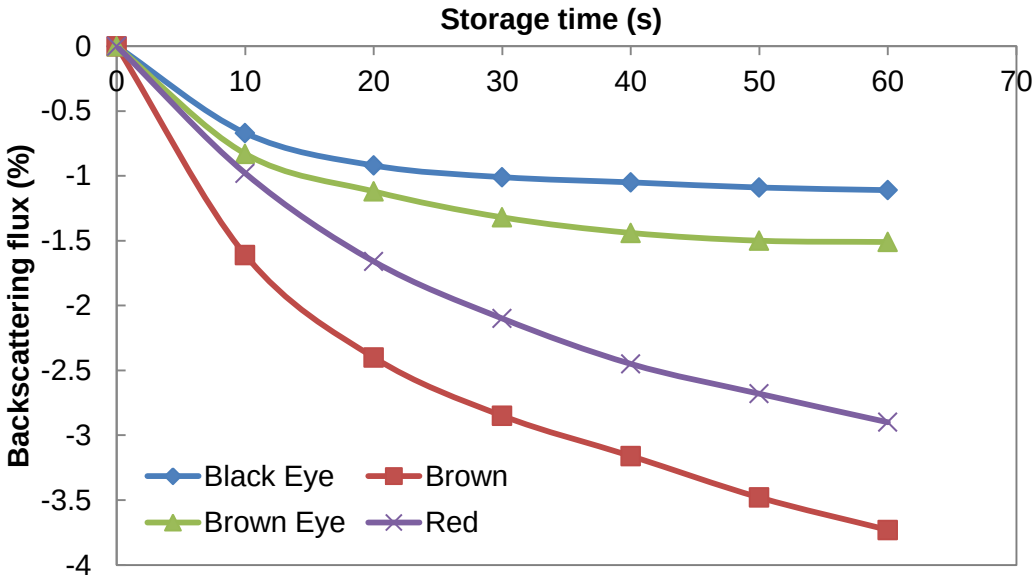

Figure 5.6 Variation in backscattering in the 20 – 40 mm zone monitored over 60 minutes for samples stored in quiescent condition at 20°C (emulsions stabilised by different varieties of Bambara groundnut fibre).

a **b** **c** **d**

Figure 5.7 Diluted orange oil beverage emulsions stabilised with 30% Bambara groundnut soluble dietary fibre (a) Black-eye (b) Brown-eye (c) Brown (d) Red.

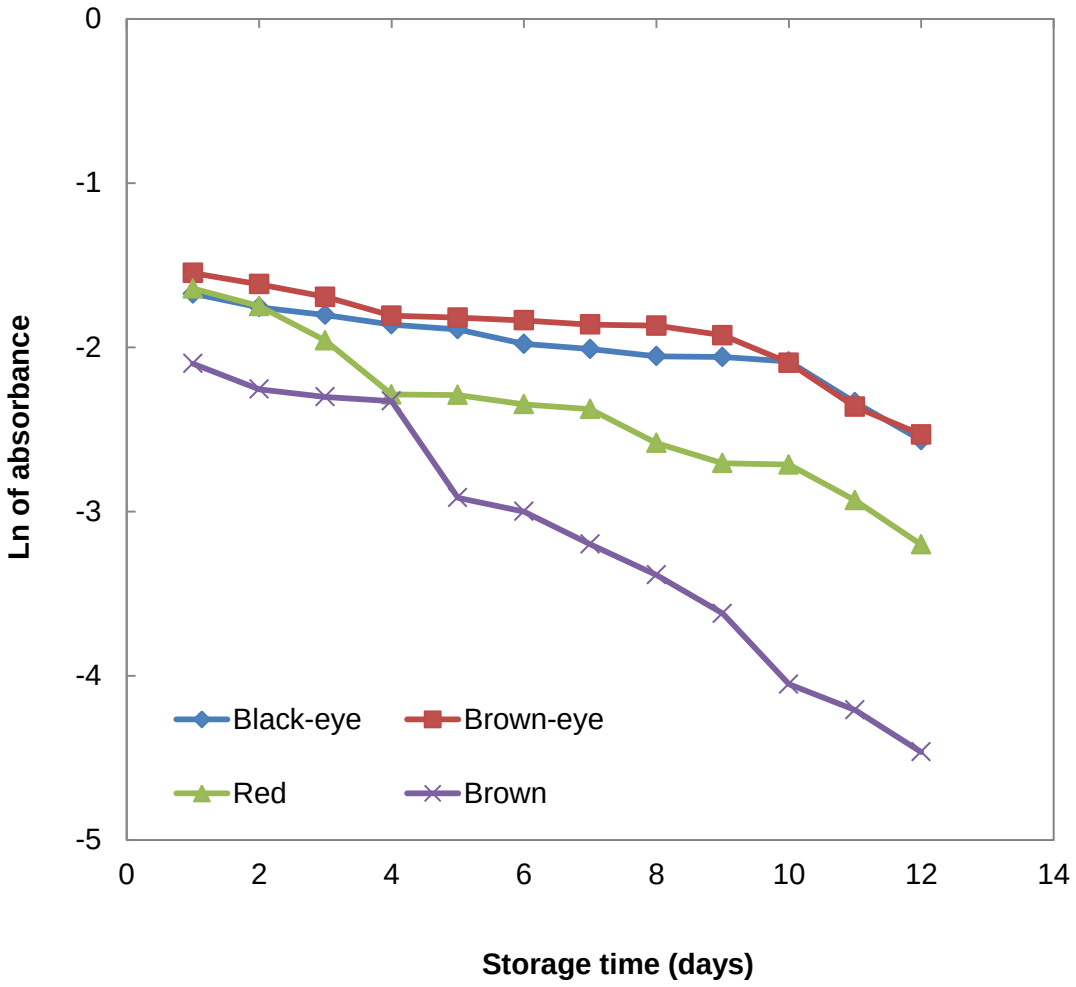

Figure 5.8 Turbidity loss of four Bambara groundnut soluble dietary fibre stabilised emulsions.

Table 5.3 Turbidity loss rate model parameters

Variety	k (day)	A	R^2
Black-eye	0.070 ± 0.00[a]	0.211 ± 0.07[a]	0.895
Brown-eye	0.084 ± 0.01[b]	0.250 ± 0.01[b]	0.862
Red	0.161 ± 0.01[c]	0.242 ± 0.02[c]	0.840
Brown	0.221 ± 0.01[d]	0.181 ± 0.01[d]	0.980

Values are mean ± standard error. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different. A: absorbance. k: turbidity loss rate. R^2: coefficient of determination.

The coefficient of determination (R^2) of all four BGN SDF stabilised emulsions was ≥ 0.840 indicating that the model could be successfully used to describe the turbidity loss rate.

The turbidity loss rates of BGN SDF stabilised emulsions were assessed under accelerated conditions so as to simulate their shelf lives in the finished products. Turbidity loss rate is a tool of assessing cloud stability of emulsions in diluted form (Mirhosseini *et al*., 2008). In this study, turbidity loss indicated that the cloudiness of all four BGN SDF stabilised emulsions decreased with storage. A decrease in turbidity of emulsions during storage has been reported by other researchers (Mohagheghi *et al*., 2011; Gharibzahedi *et al*., 2012). The decrease in turbidity during storage of BGN SDF stabilised emulsions can be attributed to loss of SDF around the layers of film formed at interfacial surface (Mohagheghi, 2011). Loss of turbidity could also have been due to factors such as flocculation, coalescence and aggregation which would have resulted in an increase in average droplet sizes which would consequently lead to destabilisation of the emulsions (Mirhosseini *et al*., 2008; Mohagheghi *et al*., 2011; Homayoonfal *et al.*, 2015). A lower amount of turbidity loss is an indication of higher stability and suggests that the emulsion in question would maintain its cloudiness for longer periods of time (Gharibzahedi *et al*., 2012). Such behaviour would attract more consumer acceptability in products such as soft drinks (Homayoonfal *et al.*, 2015).

The results obtained in this analysis were in agreement with the results of Turbiscan analysis discussed in section 5.3.3 (page 155). Turbiscan analysis predicted that black-eye and brown-eye SDF stabilised emulsions would have the highest stability with time while brown SDF stabilised emulsion would have the least. Furthermore, black-eye and brown-eye SDF stabilised emulsions had the smallest average droplet sizes while red and brown SDF stabilised emulsions had the highest droplet sizes [section 5.3.1, page 149]. This could explain the turbidity loss rates of these emulsions because the stability of an emulsion is largely dependent on droplet sizes with smaller droplets giving more stable emulsions (Dickinson, 2008; Rezvani *et al.*, 2011; Homayoonfal *et al.*, 2015).

5.3.5 Rheological properties of BGN emulsions

1. *Time dependent rheological characterisation of BGN SDF stabilised emulsions*

The steady shear properties of the four emulsions stabilised with BGN SDFs are given in Figure 5.9. The rheograms of black-eye and brown-eye SDF stabilised emulsions showed smaller hysteresis loop areas while brown and red SDF stabilised emulsions showed wider hysteresis loop areas.

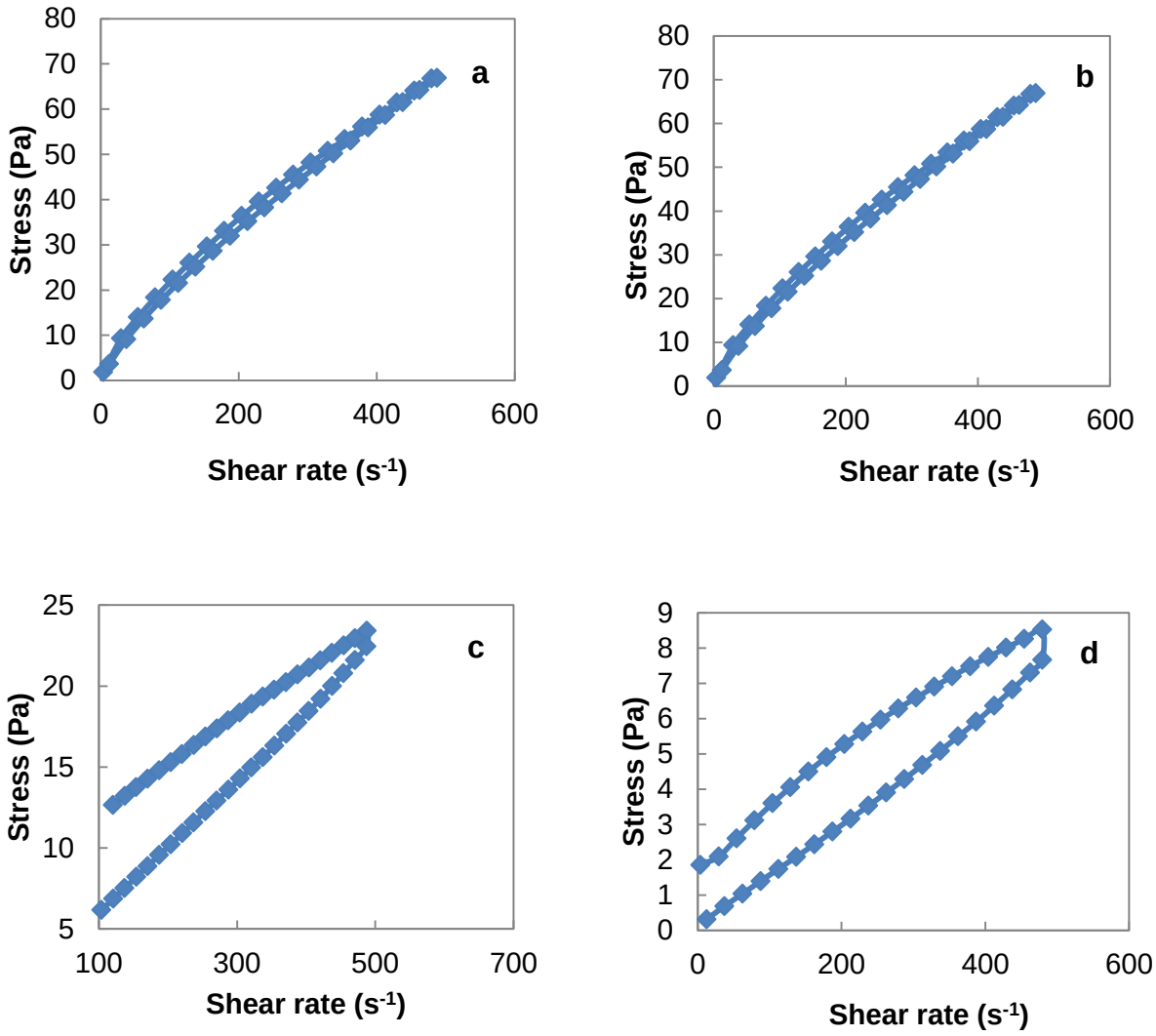

Figure 5.9 Time dependent properties of Bambara groundnut soluble dietary fibre stabilised emulsions (a) Black-eye (b) Brown-eye (c) brown (d) Red.

The presence of areas between the backward sweep and forward sweep (Figure 5.9) indicated that all four emulsions were time dependent and thixotropic in nature (Adeyi, 2014).

Figures 5.9a and 5.9b indicated that black-eye and brown-eye SDF stabilised emulsions maintained their structure over time at high shear rates as evidenced by the smaller hysteresis loop area (Table 5.4) and hence could be said to be very stable. Red and brown SDF stabilised emulsions were shown to be relatively unstable, their structures dissociated greatly at a shear rate of 500 s^{-1} (Figures 5.9c and 5.9d). The results of this study are in agreement with those obtained in section 3.4.8 (page 84) where it was established that red and brown SDFs had the least stable structures and were greatly disintegrated with increase in shear rate with time. The similarity in the results obtained in Chapter 3 and those obtained in the present analysis is an indication that BGN SDFs imparted their rheological properties on the respective emulsions they stabilised.

Table 5.4 gives the hysteresis loop areas of the four BGN SDF stabilised emulsions in the range 9.31 Pas^{-1} (black-eye SDF stabilised emulsion) to 238.61 Pas^{-1} (brown SDF stabilised emulsion). All four SDF stabilised emulsions differed significantly ($p < 0.05$) in their hysteresis loop areas. The hysteresis loop area shows the extent of breakdown within a material with increase in shear as is an index of unit of time and energy per unit volume required to eliminate the influence of time in the flow behaviour (Koocheki & Razavi, 2009). As such, larger hysteresis loop areas are indicative of more extensive damage (Tarrega *et al.*, 2004). Hence, it could be deduced that the structure of black-eye SDF stabilised emulsion maintained its integrity the most with increase in shear while that of brown SDF stabilised emulsion was disintegrated the most during shearing.

2. *Effect of BGN SDFs on apparent viscosity of orange oil emulsion*

The coefficient of determination (R^2) of all four emulsions was above 0.99 which indicated that Power law could be accurately employed for predicting the intrinsic rheological properties of the emulsions. Table 5.5 shows the Power law model parameters for the four BGN SDF stabilised emulsions. The mean consistency coefficient (K) ranged from 0.28 to 1.46 Pas^n. The consistency coefficient of all four SDF stabilised emulsions differed significantly ($p < 0.05$). The flow behaviour index was in the range 0.45 to 0.73 which was an indication of shear thinning behaviour since all the values were below 1 (Rezvani *et al.*, 2011; Lim *et al*., 2011). The flow behaviour index of brown-eye SDF stabilised emulsion was statistically ($p > 0.05$) similar to that of black-eye and red SDF stabilised emulsions but significantly ($p < 0.05$) different from that of brown SDF stabilised emulsion. Red and brown SDF stabilised emulsions did not differ significantly ($p > 0.05$) in respect to flow behaviour index.

Table 5.4 Hysteresis loop areas for four Bambara groundnut soluble dietary fibre stabilised emulsions

Variety	Integrating area for upward curve	Integrating area for downward curve	Hysteresis loop area (Pas^{-1})
Black-eye	880.03	870.72	9.31 ± 0.03^{a}
Brown-eye	777.54	768.06	9.48 ± 0.07^{b}
Red	330.22	222.64	107.58 ± 0.03^{c}
Brown	978.13	739.52	238.61 ± 0.49^{d}

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different.

Table 5.5 Power law model parameters for four Bambara groundnut soluble dietary fibre stabilised emulsions

Variety	K ($Pa \cdot s^{n)}$	n	R^2
Black-eye	0.74 ± 0.99^{a}	0.73 ± 0.03^{a}	0.9994
Brown-eye	1.46 ± 0.99^{b}	0.63 ± 0.06^{ab}	0.9961
Red	0.28 ± 0.71^{c}	0.55 ± 0.06^{bc}	0.9993
Brown	1.41 ± 0.57^{b}	0.45 ± 0.07^{c}	0.9949

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly [$p > 0.05$] different. K: consistency coefficient. n: flow behaviour index. R^2: coefficient of determination.

The consistency coefficient (K) describes the viscosity of a system with higher values indicating more viscous solutions and the flow behaviour index (n) is a measure of rigidity or reluctance of a fluid to flow. An increase in consistency coefficient results in a decrease in flow behaviour index. Red SDF stabilised emulsion showed the least viscosity and brown-eye SDF stabilised emulsion showed the highest viscosity. In section 3.4.8 (page 88), the apparent viscosity of BGN SDFs in solution was assessed and red SDF solutions showed the least viscosity while brown-eye SDF solutions showed the highest viscosity. This observation indicated that the behaviour of the emulsions was affected by BGN SDFs.

Figure 5.10 shows the change in apparent viscosity in all four emulsions with increase in shear rate. Black-eye and brown SDF stabilised emulsions had the highest initial apparent viscosity (1.58 Pas^{-1}) while red SDF stabilised emulsion had the lowest initial viscosity (0.59 Pas^{-1}). With increase in shear, black-eye SDF stabilised emulsion maintained its viscosity more than brown SDF stabilised emulsion reaching a viscosity of 0.29 Pas^{-1} at a shear of 500 s^{-1} while brown SDF stabilised emulsion deteriorated to 0.08 Pas^{-1} at 500 s^{-1}. This indicated that although brown and black-eye SDF stabilised emulsion impart the same initial viscosity, with increase in shear, brown SDF stabilised emulsion weakened and became less viscous compared to black-eye SDF stabilised emulsion.

All four emulsions exhibited non-Newtonian behaviour; a decrease in apparent viscosity was observed with increase in shear rate. This is a typical shear thinning behaviour observed in various food emulsions such as walnut O/W emulsion (Nikovska, 2010), low fat emulsion stabilised with a mixture of xanthan/guar gum (Lorenzo *et al.*, 2008), salad dressing type emulsion stabilised with a mixture of xanthan/guar gum (Gallegos *et al.*, 2004) as well as most gum solutions such as slurries, fruit juice concentrates, ketchup, syrup and molasses (Phillips & Williams, 2000). Section 3.4.8 (pages 84 – 93) described the viscosity of the individual BGN SDF in solution and the viscosities of the individual fibres in solution were similar to the rheology of the respective emulsions. Therefore, it can be deduced that BGN SDFs were responsible for the rheological behaviour of the emulsion systems.

Gallegos *et al.* (2004) stated that, water soluble polysaccharides act as thickening or structuring agents in the continuous phase of emulsion. The authors further explained that these polysaccharides decrease the extension of creaming and flocculation by decreasing the mobility of oil droplets. From this study, it can be deduced that black-eye and brown-eye SDFs efficiently played these roles. The shear thinning characteristics of BGN SDF stabilised emulsions suggested that, when stirred at slow speed or during shelf storage, BGN SDF will be randomly arranged and partially aligned in solution, resulting in a higher viscosity.

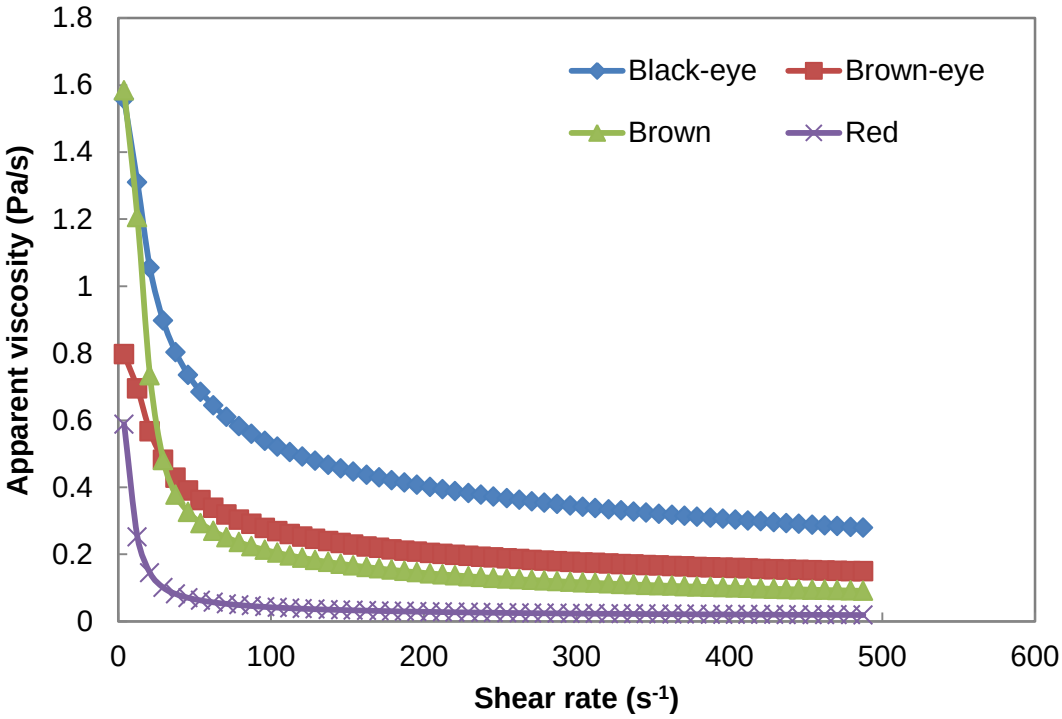

Figure 5.10 Effect of constant shear rate on the apparent viscosity of four Bambara groundnut soluble dietary fibre stabilised emulsions.

By increasing shear rate, these molecules would be expected to align themselves in a more parallel manner thereby offering less resistance to flow, consequently, resulting in a lower viscosity (Phillips & Williams, 2000).

3. *Viscoelastic properties of BGN soluble dietary fibre stabilised emulsions*

Figure 5.11 show the amplitude sweep rheograms of SDF stabilised emulsions. Black-eye (Figure 5.11a) and brown-eye (Figure 5.11b) SDF stabilised emulsions had G' (storage modulus) positioned above G'' (loss modulus). At low amplitudes storage modulus (G') and loss modulus (G'') were almost equal (overlapping) in black-eye SDF stabilised emulsion however at higher amplitudes storage modulus storage modulus (G') was evidently above loss modulus (G''). In brown-eye SDF stabilised emulsion, storage modulus (G') was clearly above loss modulus (G'') both at low and high amplitudes. In brown (Figure 5.11c) and red (Figure 5.11d) SDF stabilised emulsions, loss modulus (G'') was positioned above storage modulus (G') with red showing a wider difference between the two moduli.

The presence of both storage modulus (G') and loss modulus (G'') in all four emulsions indicated the presence of both elasticity and viscosity within all the emulsion systems. The position of these moduli however indicated which modulus was dominant in each emulsion system. Mezger (2006) explained that the position of storage modulus (G') above loss modulus (G'') and the position of loss modulus (G'') above storage modulus (G') indicated a stable and unstable system, respectively. The unstable system would most likely exhibit a more liquid character. Based on the position of storage modulus (G') and loss modulus (G'') on the rheograms, it could be deduced that black-eye and brown-eye SDF stabilised emulsions would be stable while red and brown SDF stabilised emulsions would be unstable. The results obtained in this study were in agreement with those obtained in the oscillatory experiments of the individual SDFs [Section 3.4.8, page 93] where black-eye and brown-eye SDFs exhibited storage modulus (G') positioned above loss modulus (G'') while brown and red SDFs exhibited loss modulus (G'') positioned above storage modulus (G').

It can then be argued that black-eye and brown-eye SDFs are capable for imparting elastic properties on orange oil beverage emulsions. The emulsions studied in this chapter were concentrated (30% BGN SDF). Derkach (2009) stated that concentrated emulsions have an elastic behaviour and as a result storage modulus (G') tends to dominate loss modulus (G''). This statement was accepted for black-eye and brown-eye SDF stabilised emulsions but was rejected for brown and red SDF stabilised emulsions.

In chapter 3 (Table 3.6), black-eye and brown-eye SDFs were shown to have a high total sugar composition. The higher sugar composition could be an indication that these two

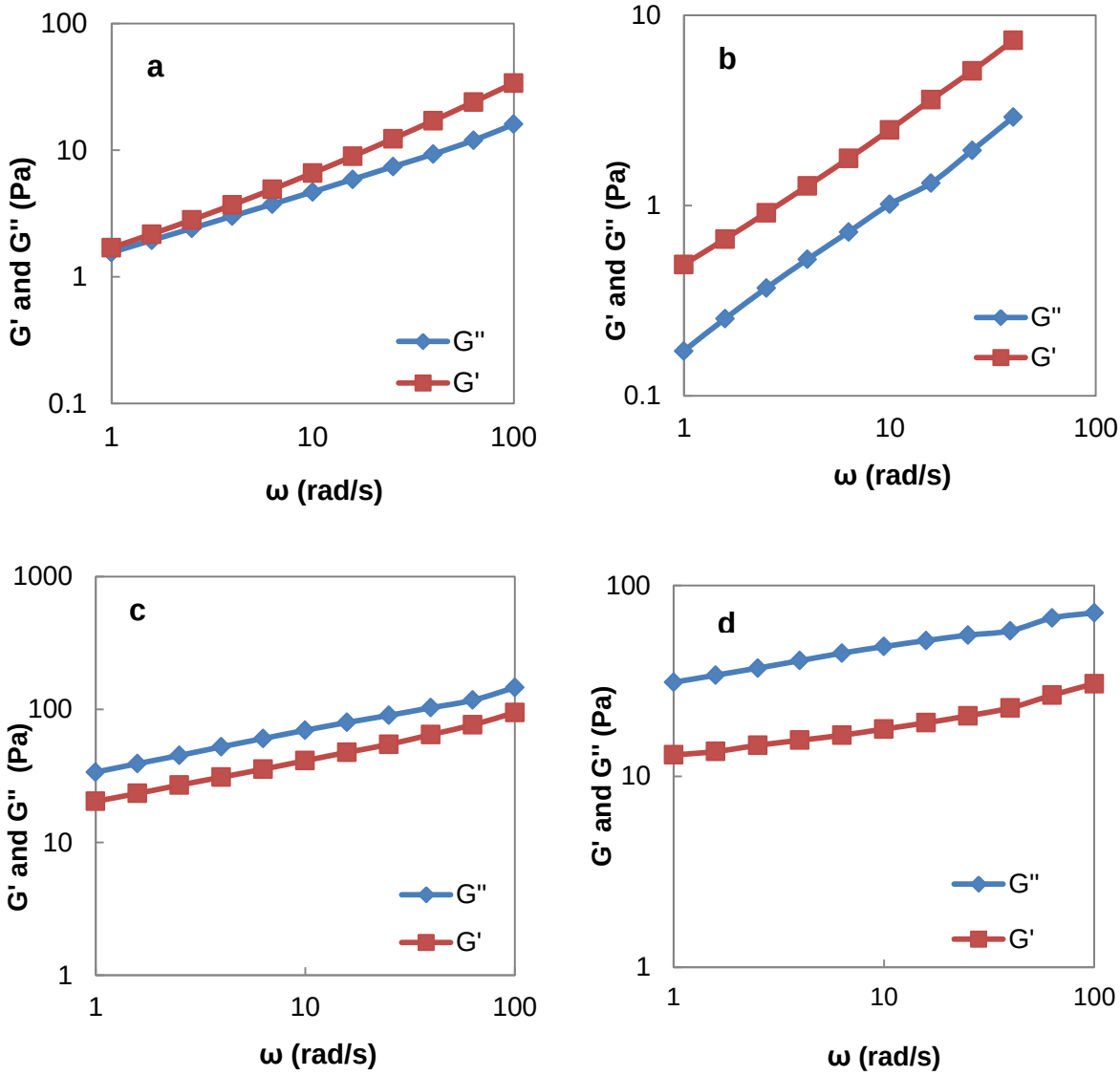

Figure 5.11 Viscoelastic properties of Bambara groundnut soluble dietary fibre stabilised emulsion (a) Black-eye (b) Brown-eye (c) Brown (d) Red. G': storage modulus. G'': loss modulus.

SDFs are superior in carbohydrate composition and these carbohydrates could be responsible for their oscillatory behaviour.

Gallegos *et al.* (2004) recorded that the presence of sugars in casseinate-stabilised emulsion resulted in an increase in elastic modulus. Gabriel *et al*. (2013) reported that there is a need for natural emulsifiers and stabilisers in the food industry. BGN SDFs, specifically those from the black-eye and brown-eye varieties could play an important role in replacing artificial stabilisers in food products. In addition they will have the advantage of being non-toxic, affordable and contribute antioxidant properties.

It is of importance to characterise the rheological properties of food systems such as emulsions so as to establish the relationship between the structure of the system and flow behaviour. This helps to relate the physical properties with sensory properties of food systems; such knowledge is of importance in processes such as mixing, handling, storage and distribution (Weiss, 2008). The results of this study suggested that the mixing, processability and handling of BGN SDF stabilised emulsions will be easy as the viscosity of the emulsions will decrease with increasing mixing. In addition, pumping of the emulsion, such as in packaging, will be easier due to the less resistance to flow behaviour of the emulsions with exertion of force. Hence, the emulsions will not clog pumps and pipes. Hence, emulsion rheology affects the textural and processability of products as well as their mouth feel (Weiss, 2008). Flow characteristics of emulsions also affect mouthfeel and processability. Less pseudoplastic emulsions exhibit 'long' flow and thus tend to be slimy while more pseudoplastic emulsions exhibit 'short' flow and thus tend to be less or non-slimy (Phillips & Williams, 2000). The pseudoplastic nature of BGN SDF stabilised emulsions suggested that these emulsions would be expected to be non-slimy in the mouth thus having a positive effect on their sensory properties.

5.3.6 Principal components explaining variability in the stability and rheological properties of the fibre stabilised emulsions

Principal component analysis (PCA) was used to reduce variability of data among the four SDF stabilised emulsions. The suitability of data reduction by PCA was determined using factors such as high correlations between the variables (correlation matrix), significant($p < 0.05$) Bartlett's test and Kaiser-Meyer-Olkin measure (>0.6) (Diedericks, 2014). Table 5.6 shows the results of PCA of the various variables for the four BGN SDF stabilised beverage emulsions. PCA is a data reduction tool. It reduces information using fewer variables called "principal" components which account for most of the variance in the original variables (Fernandes *et al.*, 2014).

Table 5.6 Coefficient correlations between variables and components for Bambara groundnut soluble dietary fibre stabilised beverage emulsions

	Component	
	1	**2**
Droplet size ($d_{3,2}$)	0.997	0.009
Droplet size ($d_{4,3}$)	0.994	-0.012
Hysteresis (Pas^{-1})	0.983	-0.038
Backscattering (Initial BS %)	-0.970	0.205
n	-0.937	-0.032
K	0.215	0.976

K: consistency coefficient; n: flow behaviour index. $d_{3,2}$: volume-surface mean diameter. $d_{4,3}$: equivalent volume-mean diameter.

From Table 5.6, component 1 was shown to represent the volume-surface mean diameter ($d_{3,2}$) (0.997), the equivalent volume-mean diameter ($d_{4,3}$) (0.994), hysteresis loop area (0.983) as well as the consistency coefficient (K) to a lesser extent (0.215). Backscattering (BS) and flow behaviour index (n) where not associated with component 1 as shown by the large negative values (Table 5.6). Component 2 showed strong association with consistency coefficient (K) (0.976) and a lesser association with backscattering (0.205) and the volume-surface mean diameter ($d_{3,2}$) (0.009). There was no association between component 2 and the equivalent volume-mean diameter ($d_{4,3}$), n and hysteresis loop areas (Table 5.6).

Figure 5.12 shows the score plot showing variations among the BGN SDF stabilised emulsions. Component 1 accounted for 79.1% of variability and component 2 accounted for 17.2% of variability. The cumulative variation of the two components amounted to 96.3%. The positive matrix of component 1 showed a relationship between red and brown SDF stabilised emulsions and hysteresis loop area, consistency coefficient (K), the volume-surface mean diameter ($d_{3,2}$) and the equivalent volume-mean diameter ($d_{4,3}$). On the negative matrix of component 1 were the black-eye and brown-eye SDF stabilised emulsions associated with backscattering. Component 2 separated the brown-eye and brown SDF stabilised emulsions associated with hysteresis loop area, consistency coefficient (K), the volume-surface mean diameter ($d_{3,2}$) and the equivalent volume-mean diameter ($d_{4,3}$) from the black-eye and red SDF stabilised emulsions (Figure 5.12) associated with backscattering and the equivalent volume-mean diameter ($d_{4,3}$). Knowledge of the properties of emulsions analysed using PCA in this study are of importance in determining the behaviour of the emulsions in various systems as well as in predicting characteristics such as their shelf life.

5.4 Conclusions

This study demonstrated that BGN SDFs can be successfully used as beverage emulsion stabilisers. The rheological properties, emulsion microstructures, droplet sizes and droplet size distributions, stabilities as well as turbidity loss of BGN SDF stabilised emulsions all indicated that black-eye and brown-eye SDFs form more stable emulsions while brown and red SDFs form less stable emulsions. All BGN stabilised emulsions are non-Newtonian, pseudoplastic fluids. BGN SDF stabilised emulsions are stable to creaming and destabilised mainly by phenomenon involving oil droplet aggregation. Each emulsion behaves slightly different due to compositional differences and physicochemical properties such as oil binding capacity and water binding capacity. Turbidity loss rate was dependent on the average droplet sizes of the emulsions, with emulsions having smaller droplets exhibiting the least turbidity. The stability of BGN SDF stabilised emulsions is dependent on the rheological behaviour of the emulsions.

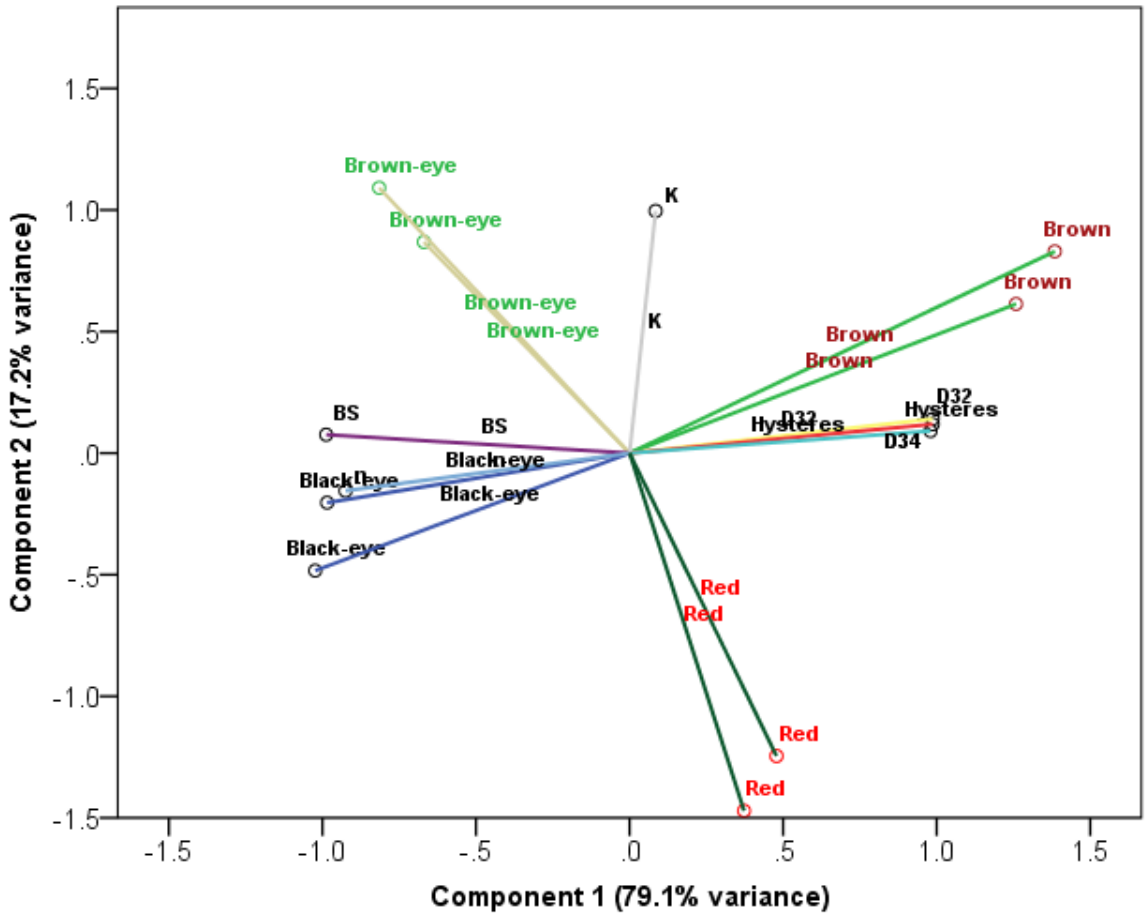

Figure 5.12 Score plot showing variations of Bambara groundnut soluble dietary fibre stabilised emulsions with respect to components 1 and 2.

References

Abismail, B., Canselier, J.P., Wilheim, A.M., Delmas, H. & Gourdon, C. (1999). Emulsification by ultrasound: drop size distribution and stability. *Ultrasonic Sonochemistry,* **6**(1-2), 75 – 83.

Acton, A. (2012) Beverage emulsions as functions of main emulsion components. In: *Issues in Food production, Processing and Preparation*. Atlanta: Scholarly Editions.

Alvarez-Cerimedo, M.S., Iriart, C.H., Candal, R.J. & Herrera, M.L. (2010). Structure of lipid emulsions formulated with sodium caseinate. *Food Research International,* **43**(5), 1482 – 1493.

Adeyi, O. (2014). Effect of Bambara Groundnut flour on the stability and rheological properties of oil-in-water emulsion. phD Thesis, Cape Peninsula University of Technology.

Aveyard, R., Bink, B.P. & Clint, J.H. (2002). Emulsions stabilised solely by colloidal particles. *Advances in Colloid and Interface Science,* **100 – 102**, 503 – 546.

Behrend, O., Ax, K. & Schubert, H. (2000). Influence of continuous phase viscosity on emulsification by ultrasound. *Ultasonic Sonochemistry,* **7**(2), 77 – 85.

Blijdenstein, T.B.J. (2004). Serum separation and structure of depletion- and bridging-flocculated emulsions: a comparison. *Colloids and Surfaces. A Physicochemical and Engineering Aspects*, **245**, 1 – 3.

Chanamai, R. & McClements, D.J. (2000). Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *Journal of Agricultural and Food Chemistry*, **48**, 5561.

Chanamai, R., Herrmann, N. & McClements, D.J. (2000). Probing floc structure by ultrasonic spectroscopy, viscometry and creaming measurements. *Langmuir,* **16**, 5884.

Cheong, K.W., Tan, C.P., Mirhosseini, H., Joanne-Kam, W.Y., Hamid, N.S.A. & Basri, M. (2014). The effect of prime emulsion components as a function of headspace concentration of soursop flavor compounds. *Chemistry Central Journal*, **8**(23), 1 – 11.

Desplanques, S., Renou, F., Grisel. M. & Malhiac, C. (2012). Impact of chemical composition of xanthan and acacia gums on the emulsification and stability of oil-in-water emulsions. *Food Hydrocolloids*, **27**, 401 - 410.

Derkach, S.R. (2009). Rheology of emulsions. *Advances in Colloid and Interface Science,* **151**, 1 - 23.

Dhingra, D., Michael, M.,Rajput, H. & Patil, R.T. (2011). Dietary fibre in foods: A Review. *Journal of Food Science and Technology,* **49**(3), 255 – 266.

Dickinson, E. (2008). Interfacial structure and stability of food emulsions as affected by protein-polysaccharide interactions. *Soft Matter,* **4**, 932 – 942.

Diedericks, C.F. (2014). Functional properties of Bambara groundnut (*Vigna Subterranea* (L) Verdc.) non-starch polysaccharides in model and food systems. Master of Technology Thesis, Cape Peninsula University of Technology.

Fernandes, J., Su, W., Rahat-Rozenbloom, S., Wolever, T.M.S. & Comelli, E.M. (2014). Adiposity, gut microbiota and faecal short chain linked in adult humans. *Nutrition & Diabetes,* **4**, 2044 – 2052.

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 Biological, Veterinary, Agricultural and Food Engineering,* **7**(11), 724-732.

Gallegos, C., Franco, J.M. & Partal, P. (2004). Rheology of food dispersions. *The British Society of Rheology,* 19 – 65.

Gharibzahedi, S.M.T., Mousavi, S.M., Hamedi, M. & Ghasemlou, M. (2012). Response surface modeling for optimization of formulation variables and physical stability assessment of walnut oil-in-water beverage emulsions. *Food Hydrocolloids*, **26**, 293 – 301.

Harnsilawat, T., Pongsawatmanit, R. & McClements, D.J. (2006). Stabilization of Model Beverage Cloud Emulsions Using Protein−Polysaccharide Electrostatic Complexes Formed at the Oil−Water Interface. *Journal of Agricultural and Food Chemistry,* **54**(15), 5540 – 5547.

Homayoonfal, M., Khodaiyan, F. & Mousavi, M. (2015). Modelling and optimising of physicochemical features of walnut-oil beverage emulsions by implementation of response surface methodology: Effect of preparation conditions on emulsion stability. *Food Chemistry,* **174**, 649 – 659.

Koocheki, A., & Razavi, S. M. A. (2009). Effect of concentration and temperature on flow properties of *Alyssum homolocarpum* seed gum solutions: assessment of time dependency and thixotropy. *Food Biophysics*, **4**, 353 - 364.

IBM Corp. (2013). IBM Statistical Package for the Social Science (SPSS), Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

Kumar, R., Kumar, M.S. & Mahadevan, N. (2012). Multiple emulsions: A Review. *International Journal of Recent Advances in Pharmaceutical Research*, **2**(1), 9 – 19.

Lim, S.S., Baik, M.Y., Decker, E.A., Henson, L., Popplewell, L.M., McClements, D.J. & Choi, S.J. (2011). Stabilization of orange oil-in-water emulsions: A new role for ester gum as an Ostwald ripening inhibitor. *Food Chemistry,* **128**(4), 1023 – 1028.

Lorenzo, G., Zaritzky, N. & Califano, A. (2008). Modeling Rheological Properties of Low-in-fat O/W emulsions stabilized with Xanthan/guar mixtures. *Food Research International,* **41**, 452 - 494.

Mengual, O., Meunier, G., Cayre, I., Puech, K. & Snabre, P. (1999). TURBISCAN MA 2000: Multiple light scattering measurement for concentrated emulsion and suspension instability analysis. *Talanta,* **50**(2), 445 – 456.

Mezger, T.G. (2006). The rheology handbook for users of rotational and oscillatory rheometers. 2nd ed., Hannover: Vincentz Network.

Mirhosseini, H., Tan, C.P., Hamid, N.S.A. & Yusof, S. (2008). Effect of Arabic gum, xanthan gum and orange oil on flavor release from diluted orange beverage emulsion, *Food Chemistry*, **107**(3), 1161 – 1172.

Mohagheghi, M., Rezaei, K., Labbafi, M. & Mousavi, S.M.E. (2011). Pomegranate seed oil as a functional ingredient in beverages. European Journal of Lipid Science and Technology, **113**, 730 - 736

Nikovska, K. (2010). Oxidative stability and rheological properties of oil-in-water Emulsions with Walnut Oil. *Advance Journal of Food Science and Technology* 2: 172 - 177.

Phillips, G.O. & Williams, P.A. (2000). Hydrocolloids and emulsion stability. In: *Handbook of Hydrocolloids.* Cambridge: Woodhead Publishing Ltd.

Reiner, S.J., Reineccius, G.A. & Peppard, T.L. (2010). A Comparison of the Stability of Beverage Cloud Emulsions Formulated with Different Gum Acacia- and Starch-Based Emulsifiers. *Journal of Food Science*, **75**, 236 – 246.

Rezvani, E., Taherian, A.R. & Schleining, G. (2011).Physical Stability of Beverage Emulsions as Influences of Orange Oil, Tragacanth and Arabic Gums Concentrations *LWT – Food Science and Technology,* **48**(1), 82 – 88.

Sahin, S. & Sumnu, S.G. (2006). Rheological properties of foods. In: *Physical properties of foods*. Pp. 39 - 105. Turkey: Springer.

Taherian, A., Britten, m., Sabik, H. & Fustier. (2010). Ability of whey protein isolate and/or fish gelatin to inhibit physical separation and lipid oxidation in fish oil-in-water beverage emulsion. *Food Hydrocolloids,* **25**(5), 868 – 878.

Tarrega, A., Duran, L. & Castell, E. (2004). Flow behavior of semi-solid dairy desserts, Effect of temperature. *International Dairy Journal,* **14**, 345-353.

Tcholakova, S., Denkov, N.D. & Danner, T. (2004). Role of surfactant type and concentration for the mean drop size during emulsification in turbulent flow. *Langmuir*, **20**, 7444 - 7458.

Weiss, J. (2008). Emulsion rheology and textural analysis. Emulsion workshop, 13 - 14 November 2008. University of Hohenheim, Germany.

Yadav, M.P., Johnston, D.B., Hotchkiss, A.T. & Hicks, K.B. (2007). Corn fibre gum: A potential gum arabic replacer for beverage flavor emulsification. *Food Hydrocolloids*, **21**(7), 1022 – 1030.

CHAPTER SIX
GENERAL SUMMARY AND CONCLUSIONS

This thesis reported the extraction of dietary fibres from Bambara groundnut (BGN) (*Vigna subterranea* (L.) Verdc) using the wet milling method. The aim of this study was to find a cheaper alternative of extracting BGN soluble and insoluble dietary fibres to the enzymatic-gravimetric method. The objectives of the study were to characterise the physicochemical properties of BGN non-starch polysaccharides from wet milling and investigate their prebiotic and beverage emulsion stabilising properties. Dietary fibres were extracted from four varieties of BGN namely the black-eye, brown-eye, brown and red.

The first objective of chapter three was to extract soluble and insoluble dietary fibres from whole seeds of BGN using the wet milling method as an alternative to the enzymatic-gravimetric method. The wet milling method was successfully applied in the extraction of soluble and insoluble dietary fibres from BGN whole seeds (cotyledons and hulls). The hypotheses that the extraction of SDFs and IDFs from BGN will be possible using the wet milling method, was accepted.

The second objective of chapter three was to evaluate the physicochemical of BGN dietary fibres. The hydration properties (water holding capacities and swelling capacities), oil binding capacities, antioxidant properties (hydrolysable polyphenols and condensed tannins), densities (bulk and direct), total sugar and uronic acid content of BGN dietary fibres revealed their potential for various applications in food systems. Furthermore, BGN dietary fibres were thermally stable indicating that they would withstand elevated processing temperatures in the food industry such as those applied in baking. Black-eye and brown-eye dietary fibres have superior physicochemical properties compared to the dietary fibres from the brown and red varieties as they had higher swelling capacities, water holding capacities, oil binding capacities, antioxidant content, total sugar content as well as superior rheological and thermal stabilities. The physicochemical properties of BGN dietary fibres make them valuable to the food industry as potential fortifiers, thickening agents, stabilisers and fat binders amongst other functions. Similarities in physicochemical properties between BGN dietary fibres and other commercial dietary fibres were observed indicating the suitability of BGN dietary fibres as alternatives to these fibres. Therefore, BGN dietary fibres would be expected to compete successfully with other fibres in the market.

The third objective of chapter three was to evaluate the rheological properties of BGN SDFs solutions (4 - 14%). All BGN SDFs exhibited shear thinning (pseudoplastic) behaviour and thixotrophy. This is of importance in food systems as these fibres would be expected to exhibit ‘short’ flow and therefore be non-slimy in the mouth thus having a positive effect on their sensory properties. Higher concentrations of BGN SDFs had higher viscosities indicating that where high viscosity is desired in food systems, higher concentrations of BGN

SDFs would be required. The structural breakdown of BGN SDFs as indicated by the presence of hysteresis loop areas showed black-eye and brown-eye SDFs as the most stable structures.

The fourth objective of chapter three was to compare the cost of the wet milling and the enzymatic-gravimetric method. The cost of BGN dietary fibre extraction from the wet milling method was estimated to cost ZAR9130.48/kg while from the enzymatic-gravimetric method was estimated to cost ZAR26358.57/kg. The huge difference in the cost of the two methods suggested that the wet milling would be a more preferable alternative in BGN dietary fibre extraction in terms of cost. The good yield coupled with less cost of the wet milling method makes it a suitable alternative to the enzymatic-gravimetric method. The hypotheses that the wet milling method would be relatively cheaper and simpler than the enzymatic-gravimetric method while producing fibres of a similar quality was accepted.

Chapter four aimed to investigate the prebiotic properties of BGN SDFs employing pure cultures of *Bifidobacterium* spp. as probiotics. All four BGN SDFs supported the growth of *B. breve, B. bifidum* and *B. animalis* subsp. *animalis* producing short chain fatty acids (SCFAs) (acetic and propionic acids). The quantities of SCFAs agreed fairly well with those previously reported by other researchers and in some instances were higher. The growth rates of *Bifidobacterium* spp. also increased in the presence of BGN SDFs as was explained using the kinetic parameters of Gompertz equation. Consequently, BGN SDFs were concluded to be prebiotic in nature and could be used as fortifying agents in foods, increasing the market value of those products. *Bifidobacterium* spp. are abundant in the intestinal microflora of infants and therefore the ability of *Bifidobacterium* spp to ferment BGN SDFs makes these fibres useful potential ingredients in infant foods. It can be concluded that, BGN SDFs will support the growth of *Bifidobacterium* spp. in the human colon. The hypothesis that BGN soluble dietary fibres will support the growth of all three *Bifidobacterium* spp. was accepted.

The aim of Chapter five was to evaluate BGN SDFs as potential orange oil beverage emulsion stabilisers with a view to provide the beverage industry with a low cost, alternative stabiliser. The effects of 15 – 30% (w/w) brown SDF and 6 – 10% (w/w) orange oil were evaluated and a combination of 30% SDF and 6% orange oil were found to be the optimum combination giving the highest stability. In the current study, this combination was adopted and applied using four varieties of BGN SDFs (black-eye, brown-eye, brown and red). All four BGN SDFs greatly indicated their potential in stabilising orange oil beverage emulsions both in concentrated and diluted forms. BGN SDF stabilised emulsions exhibited shear thinning behaviour and thixotrophy. Constrained non-linear regression algorithms explained the turbidity loss rates of the emulsions. BGN SDF stabilised emulsions were shown to be stable to creaming and destabilised mainly by phenomenon involving oil droplet aggregation.

Black-eye SDF stabilised emulsion had the least turbidity loss rate (k) (0.070/day) and brown SDF stabilised emulsion had the highest turbidity loss rate (k) (0.221/day). Considering the rheological properties, emulsion microstructures, droplet sizes and droplet size distributions, stabilities and turbidity loss, black-eye and brown-eye SDF stabilised emulsions are more stable than brown and red SDF stabilised emulsions. Principal component analysis showed association of brown and red SDF stabilised emulsions with higher volume-surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter ($d_{4,3}$) values as well as larger hysteresis loop areas. The following conclusions can therefore be drawn from this study:

1. The wet milling method is a suitable alternative of BGN dietary fibre extraction to the enzymatic-gravimetric method in terms of cost, handling and BGN dietary fibre yield.
2. The physicochemical, rheological and other functional properties of BGN varied with variety:

2.1 Black-eye and brown-eye dietary fibres have superior physicochemical properties compared to the dietary fibres from the brown and red varieties.

2.2 The physicochemical properties of BGN dietary fibres make them valuable to the food industry as potential fortifiers, thickening agents, stabilisers and fat binders amongst other functions.

2.3 The thermal stabilities of BGN dietary fibres make them suitable for high temperature processes in the food indeustry.

2.4 BGN SDFs have prebiotic properties and will support the growth of *Bifidobacterium* spp. in the human colon.

2.5 Black-eye, brown-eye, brown and red BGN SDFs have the ability to stabilise both concentrated and diluted orange-oil beverage emulsions.

2.6 BGN SDF stabilised emulsions are stable to creaming and destabilised mainly by phenomenon involving oil droplet aggregation.

2.7 Emulsions stabilised with black-eye and brown-eye SDFs are more stable than those established with red and brown SDFs.

Outputs from this study include the following:

1. *Peer Reviewed Publications*

Maphosa, Y. & Jideani, V. A. (2015). Dietary fiber extraction for human nutrition - A review. *Food Reviews International*. DOI of your paper is: 10.1080/87559129.2015.1057840 Available at: http://dx.doi.org/10.1080/87559129.2015.1057840

Maphosa, Y. & Jideani, V. A. (2016). Physicochemical characteristics of Bambara Groundnut dietary fibres extracted using wet milling. *South African Journal of Science*, **112**(1/2), 1 – 8. http://dx.doi.org/10.17159/sajs.2016/20150126

2. *Conference Proceedings*

Maphosa, Y. & Jideani, V. A. (2014). Dietary fibre extraction from plant materials - A review. U6 Consortium 2nd International Conference, Cape Town, South Africa, 6 - 10 September 2014. Pp. 26. (Paper presentation).

Maphosa, Y. & Jideani, V. A. (2014). Polyphenolic and neutral sugar composition of Bambara Groundnut non-starch polysaccharides. CPUT Postgraduate Conference, Bellville, Cape Town, South Africa, 5 November 2014. Pp 1. (Paper presentation).

Maphosa, Y. & Jideani, V. A. (2015). Stability and rheological properties of orange-oil beverage emulsion stabilised with Bambara groundnut soluble dietary fiber. Institute of Food Technologists, IFT15 International Conference, Chicago, Illinois, USA, 11 - 14 July, 2015. (Poster presentation).

Maphosa, Y. & Jideani, V. A. (2015). Rheological and thermal characteristics of Bambara groundnut non-starch polysaccharides. 21st South African Association of Food Science and Technology (SAAFoST) Biennial International Congress and Exhibition 2015, Durban, South Africa, 6 - 9 September 2015. (Paper presentation).

Maphosa, Y. & Jideani, V. A. (2016). Effect of Bambara groundnut soluble dietary fibres on the rheological properties of orange oil beverage emulsions. 4th International ISEKI_Food Conference 2016, Vienna, Austria, 6 - 8 July, 2016 (Poster presentation).

APPENDICES

Appendix A: Peer reviewed publication in the journal, *Food Reviews International* titled "Dietary fiber extraction for human nutrition - A review"

FOOD REVIEWS INTERNATIONAL
http://dx.doi.org/10.1080/87559129.2015.1057840

Taylor & Francis
Taylor & Francis Group

Dietary fiber extraction for human nutrition—a review

Yvonne Maphosa and Victoria A. Jideani

Department of Food Technology, Cape Peninsula University of Technology, Bellville, South Africa

ABSTRACT

Fiber is a mixture of nonstarch polysaccharides that resist digestion by enzymes in the gastrointestinal canal. Some known methods of extracting fiber from plant sources include dry processing, wet processing, chemical, gravimetric, enzymatic, physical, microbial, or a combination of these methods. Modified wet milling is the most cost-effective in the wet milling group, as it uses minimal chemicals, produces high purity products, and uses less water than the other methods. The purity of fibers extracted using the modified wet milling method range from 49.7% to 89.6%. An ideal extraction method should be affordable and produce fibers of high purity.

KEYWORDS

Dietary fiber; extraction methods; fiber functionality; insoluble fiber; plant materials; soluble fiber

Introduction

The beneficial effects of fiber have led to the development of a potential market for fiber-rich products.[1,2] To satisfy this market, new sources of fiber such as traditionally underutilized plants and recovered fruit and vegetable wastes are being researched. Various researches that support the various beneficial health effects of fiber have been carried out.[3–5] In addition, fiber has gained popularity as an ingredient and has been incorporated in different food products where it serves various purposes. Increased consumer awareness of the therapeutic potential of dietary fiber has also contributed to the increased search for new sources.[4,6]

Whereas methods of quantifying fiber have been largely studied, those of isolation and fractionation of fibers are limited.[35,7] Literature reveals that the determination of cellulose, hemicelluloses, and lignin have been largely carried out; however, there is limited research that has been conducted to isolate these components.(5) This review summarizes research that has been conducted on the methods applied for extracting fibers from plant material.

Definitions of fiber

Fiber is difficult to define because it includes a wide range of complex compounds.[8] Definitions of fiber differ worldwide; some are based on a physiological basis and others on analytical methods.[9,10] Traditionally, fiber has been defined as a mixture of polymeric nonstarch polysaccharides such as cellulose, hemicellulose, and pectin, which are resistant to digestion by enzymes in the gastrointestinal canal.[2,3,11–13] This definition is analogous with that given by Codex Alimentarius[14] and Elleuch et al.[5] who define fiber as

CONTACT Yvonne Maphosa yvonmaphosa@gmail.com Department of Food Technology, Cape Peninsula University of Technology, P.O. Box 1906 Bellville, 7535, South Africa.

nondigestible carbohydrates and lignin that are components of plants, including the nondigestible carbohydrates that have beneficial functional effects in the human body.

Fiber is defined as carbohydrate polymers with more than nine monomers, which are not digestible by the endogenous enzymes in the small intestine and belong to the following categories:

(1) Edible carbohydrate polymers occurring naturally in the food as consumed;
(2) Synthetic carbohydrate polymers that have been scientifically proven to have a beneficial physiological effect to health; or
(3) Carbohydrate polymers that have been obtained from food raw material by physical, enzymatic, or chemical means and that have been scientifically proven to have a beneficial physiological effect to health.[14]

Components of fiber

Fiber is composed of a mixture of plant carbohydrate polymers that make up the cell wall.[6,9,15,16] Plant cell walls are made predominantly of oligosaccharides and polysaccharides that include cellulose, hemicelluloses, pectin, gums, inulin, resistant starches, and some noncarbohydrate compounds such as waxes, cutin, saponins, phytates, polyphenols, and resistant proteins.[5,6,17–19]

Cellulose is the main component of cell walls; it is composed of linear chains of glucose molecules joined together by β(1→4) glycosidic bonds and is insoluble due to its extensive hydrogen bonding.[2,19] The structure of cellulose is given in Fig. 1a.

Pectin is made up of α(1→4)-linked polymers of D-galacturonic acid units esterified with methanol.[17] It is found in high quantities in the primary cell wall and in the intermediate laminate. Lignin consists of aromatic alcohols.[18] It is composed of complex molecules of polyphenylpropane units.[3] The structures of pectin and lignin are given in Fig. 1b and c, respectively.

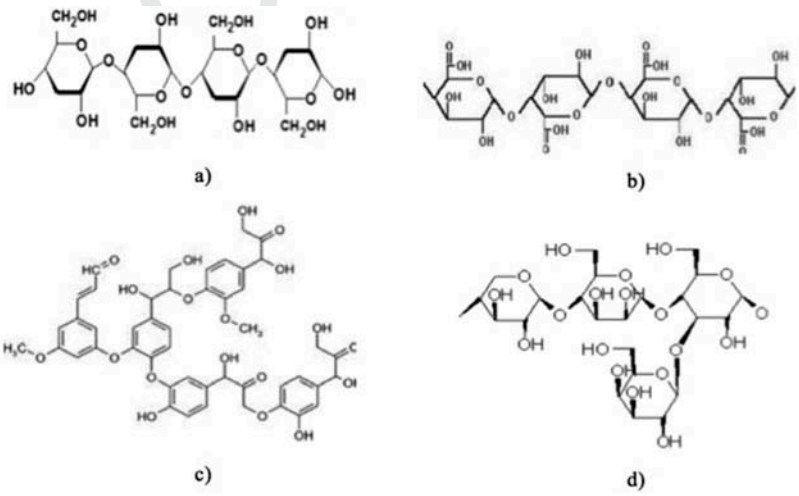

Figure 1. Structures of (a) cellulose; (b) pectin; (c) lignin; and (d) hemicellulose.

Table 1. Solubility and sources of some components of dietary fiber.

Component	Solubility	Source
Cellulose	Insoluble	Whole grains, bran, peas, root vegetables, beans family of cruciferous, apple
Pectin	Soluble	Whole grains, legumes, cabbage, root vegetables, apples
Lignin	Insoluble	Vegetables, flour
Hemicellulose	Insoluble	Bran, whole grains
Gums	Soluble	Oatmeal, legumes
Mucilage	Soluble	Additives
Inulin	Soluble	Agave, bananas, chicory, garlic, artichoke, onion, wild yam
Resistant starch	Insoluble	Uncooked potato, banana, legumes, bread, rolled oats

Note. Adapted from references 2, 13, 19, and 61.

Hemicelluloses are components of primary and secondary cell walls.[18] They are commonly found in the seed endosperm particularly in guar. Some hemicelluloses such as galactomannans consist of about 63% mannose and 35% galactose. However, most of them are linear xylose polymers with glucose, arabinose, and glucuronic acid side chains.[3] The structure of hemicellulose is given in Fig. 1d.

Gums are generally made up of hexose and pentose monomers. Mucilages, on the other hand, are synthesized by plants that contain glycoproteins.[18] Resistant proteins and resistant starches remain undigested in the small intestine. Resistant starch is divided into four groups: namely, RS1, physically inaccessible/digestion-resistant starch; RS2, ungelatinized starch granules; RS3, retrograded starch; and RS4, chemically modified starch that resists digestion.[18] Table 1 shows the different components of fiber, their solubility, and sources where they may be obtained.

Fiber classification

Fiber can be classified as dietary or functional and can further be classified according to its molecular weight as high-molecular-weight fiber or low-molecular-weight fiber.[9,20] The sum of dietary and functional fibers gives total fiber.[2,20] Dietary fiber (DF) can also be classified according to its solubility in water as soluble dietary fiber (SDF) or insoluble dietary fiber (IDF).[21] Lignin, cellulose, and some hemicelluloses typically make up the bulk of IDF, whereas pectin, inulin, β-glucans, galactomannans, gums, and other non-starch polysaccharides make up SDF.[3,15,18]

Soluble and insoluble fibers exhibit different behaviors and therefore have different physiological effects.[18] Soluble fiber presents a potential prebiotic and is associated more with cholesterol reduction in the body and decreasing the amount of glucose absorbed in the small intestines. Insoluble fiber, on the other hand, is responsible for increasing fecal bulk, intestinal regulation, and water absorption.[18] Physiologically, water absorption is of importance, as it provides laxative effects and improves peristalisis.[5,22]

Insoluble fiber is the portion of fiber that does not dissolve in water and is commonly derived from cereals.[8] Soluble fiber, on the other hand, dissolves in water and is readily fermented in the colon; fruits and vegetables are the common sources.[5,23]

The ratio of soluble to insoluble fiber, particle size, and source of the fiber are some of the attributes of importance for both functional and dietary properties.[6] A ratio of approximately 1:2 of soluble to insoluble fiber is considered acceptable for fiber destined for use as a food ingredient.[6] The soluble to insoluble fiber ratio of Bambara groundnut was reported to be 1:3.[24]

Characteristics of commercial fiber are a total dietary fiber (TDF) content of above 50%, a moisture content of less than 9%, a very low lipid and calorie content, and a neutral flavor and taste.[6] Research reveals that the characteristics of fibers are largely determined by the composition of the particular fiber, especially the ratio of the soluble to insoluble fiber fraction.[5]

Health benefits of fiber

Adequate daily intake (up to 35 g dietary fiber/day) of DF is largely recommended.[11,25] Published reports indicate the many beneficial effects of DF in the human body. These include the prevention and possible treatment of diseases and disorders such as constipation, obesity, diabetes, heart complications, piles, and some cancers.[1,3,15,16] In addition, DF, particularly soluble fiber, has the ability to lower blood cholesterol, improve glucose tolerance, and reduce glycaemic response.[3,14,26] Insoluble fibers are porous, have a low density, and have the ability to increase fecal bulk, promote normal laxation, and decrease intestinal transit.[4,5,27]

The fermentation in the colon and its interaction with the resident microflora is one of the important functions of dietary fiber. There are up to a thousand different microbial species in the human colon, and their growth is favored by the slow transit time, favorable pH, and nutrient-rich environment of the colon.[10] Saccharolytic bacteria such as *Lactobacilli* and *Bifidobacteria* are the most common, and they obtain their energy through the digestion of nonstarch polysaccharides, polyols, resistant starches, and other carbohydrates that pass the upper intestinal region undigested. The hexoses in these carbohydrates are broken down to pyruvate using various carbohydrate-hydrolyzing enzymes and produce by-products such as gasses (hydrogen, carbon dioxide, methane) and fatty acids (acetate, propionate, butyrate).[10] Energy production results in colonic bacteria increasing in mass, which in turn leads to increased fecal mass and consequently imparts a stool bulking effect. The by-products produced also play a physiologically beneficial role. Butyrates are an energy source for colonic epithelial cells and may possess a primary protective function against certain colonic disorders. Propionates, on the other hand, may play a role in lowering the hepatic production of cholesterol by interfering with its synthesis. The fermentation process and short-chain acid production results in a reduced pH, which inhibits the growth of pathogens, decreases the activity of undesirable bacterial enzymes, and reduces peptide degradation and the resultant formation of toxic compounds such as ammonia and phenolic compounds.[10]

Over the years, consumers have become more concerned about the effect of diet on health, since nutrition is associated with many diseases of lifestyle such as diabetes, obesity, and some cancers.[27,28] This includes the increased consumer awareness of the health benefits and nutritional significance of DF, which has resulted in a higher consumer demand of high fiber foods.[29] In an effort to meet this demand, the investigation of alternative sources of DF by a number of researchers has been carried out.[1,3,30–32] Fig. 2 shows some of the various beneficial effects of fiber in the human body.

The extraction of fiber, particularly from legumes, has received attention from various researchers.[3] The fiber content of most edible legumes ranges from 8% to 27.5%, with soluble fiber in the range of 3.3–13.8% expressed as dry weight. Dietary fiber values ranging from 6.9% to 9.3% for pea, broad pea, and soybean cotyledons were

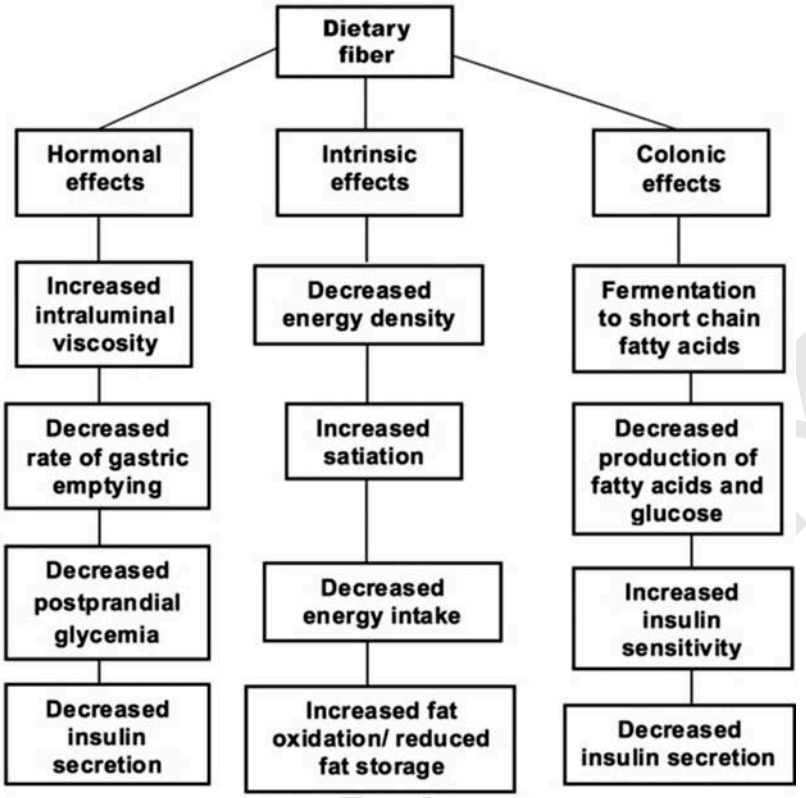

Figure 2. The various effects of dietary fiber in the human body.

reported.[11] The literature reveals that legume seeds such as Bambara groundnut have a higher amount of DF than cereals and are sources of metabolically active soluble fiber.[3,33]

The fiber content of Bambara groundnut has been documented; however, variations in the reported results exist. These variations may be attributed to differences in Bambara groundnut varieties, species, climatic conditions, type of soil grown on, processing, and determination methods used.[3,11] The Bambara groundnut dietary fiber content ranges from 5.2% to 6.4%.[33] This percentage is sufficient to fortify fiber-deficient foods. Higher values of four varieties of Bambara groundnut DF were reported with a total DF content of 17.7%, 21.0%, 23.9%, and 24.3% for brown-eye, brown, black-eye, and red varieties, respectively.[24] Bambara groundnut soluble fiber was in the range of 12.7–13.9% and insoluble fiber was in the range of 37.4–48.3% dry weight.[24] The total dietary fiber contents of various plants are given in Table 2.

Recommended intakes of dietary Fiber

Table 3 shows the recommended daily intakes (RDIs) of total dietary fiber by different food organizations. The ideal fiber intake of individuals 14 years or older should at least be

Table 2. Total dietary fiber content of various plant sources.

Source	Quantity (g/100 g)
Cereals	2.0–42
Legumes	1.9–25.5
Vegetables	0.6–16.6
Fruits	0.5–3.4
Nuts and seeds	6.0–22.3

Note. Adapted from references 2 and 65.

Table 3. Recommended daily intakes (RDIs) for individuals 14 years or older.

Organization	RDI (g)
United States Food and Drug Administration (USFDA)	25
United States Department of Agriculture (USDA)	25
American Diabetes Association (ADA)	25–35
American Dietetic Association	20–30
National Cancer Institute	20–30
Europe Union	20
World Health Organisation	25

Note. Adapted from references 8 and 13.

20 g per day (Table 3). Only a few countries report fiber intakes that adhere or are above these recommendations, with the majority of the population in developed countries consuming about 11 g/day.[18] The increase in fiber consumption for promotion of health and prevention of diseases is a critical public health goal.[6,17,28]

The RDI of total fiber intake ranges from 21 to 40 g.[18] Adults are recommended to consume up to 35 g of fiber per day with adequate fluid intake.[3] Specifically, the recommendations are broken down to 25 g/day for women younger than 50, 21 g/day for women older than 50, 38 g/day for men under 50, and 30 g/day for men over 50 years of age.[5] Total fiber intake for men is recommended as 38 g/day and 25 g/day for women.[3,18,34]

Separation and fractionation of fiber

The aim of separating fibers into their individual constituents is to isolate and quantify fractions of interest and eliminate unwanted compounds.[5,35] Various methods of fiber extraction and fractionation from various plant sources have been investigated. The extraction method, solvents, drying method, treatment intensity, and source of fiber largely affect the properties and composition of the resultant fibers.[11,17,36] The extraction method also affects the behavior of fibers in the human body and in food applications.[6]

The choice of extraction method used to isolate fibers is highly dependent on the composition of the particular fiber, its complexity, chemical nature, degree of polymerization, and the presence of oligosaccharides.[5] The choice of method, contact time, temperature, and solvent to sample ratio are some parameters that highly affect the yield.[37] Variations also exist in fibers from the same source due to agronomy variations, genetics, maturity, and climatic conditions.[37]

The basis of all the methods of extracting fiber is similar; however, the approach differs depending on the desired end product, intended application, source of fiber, and equipment used. All the methods involve fractionation; this allows for the elimination of undesired constituents.[35] Fiber can be extracted as a whole, termed total fiber, or as soluble or

insoluble fiber or as its individual constituents. Some previously researched methods of extracting fiber from plant sources include dry processing, wet processing, chemical, gravimetric, enzymatic, physical, microbial, or a combination of these methods.[38–41] Every analyst modifies a present fiber extraction procedure to suit their research, as there is no standardized fiber extraction method.[5] Some methods of fiber extraction are employed by industry, whereas others are used for experimental and research purposes. Some dietary fiber extraction methods are closely related to dietary fiber analytical methods, as shown by the research of Elleuch et al.[5] who described, amongst other methods, the enzymatic-gravimetric method as a dietary fiber analysis method, whereas Diedericks[24] applied the same method for dietary fiber extraction from Bambara groundnut.

Fiber extraction was first reported by Southgate who outlined a method of extracting cellulose and lignin fractions.[7,42] The work of Southgate triggered an interest in fiber extraction, with further research developing methods to isolate total, soluble, insoluble, and individual fiber components. Soluble fiber can be isolated using hot water coupled with EDTA to solubilize pectin and bind cations.[43] A method of insoluble fiber extraction was developed by fractionating wheat bran into hemicellulose, cellulose, and lignin using acid treatments coupled with enzymatic digestion.[44] Pectin, hemicelluloses, and cellulose were isolated using gravimetric methods that involved the use of water, 80% ethanol, and NaCl.[45] Lignin, cellulose, and hemicelluloses were isolated using chemical-enzymatic and water extractions.[46]

Dry processing methods

Dry processing methods have been applied for experimental purposes as well as industrial applications.[11] These methods involve the disintegration of seeds by milling and air classification into starch and protein fractions.[38] The flour produced during the milling process contains two distinct populations of particles, which differ in size and density.[11] To separate these two phases, a current of air is used; thus, the origin of the name "air classification." One phase consists of fine and lighter particles containing mostly starches and fibers, whereas the other phase is coarse and relatively heavier containing mainly proteins and lipids.[38]

To purify fractions, air classification is repeated on products; this, however, is a drawback, as it reduces product recovery. Advantages of dry processing methods include reduced energy and water consumption. Air classification is more efficient when used on crops that have starch as their main storage material such as peas (*P. Sativum*), faba bean (*V. Faba*), baby lima bean (*P. Lunatus*), and cowpea (*Vigna unguculata*).[11,38]

Wet processing methods

Wet milling methods all use water for fiber extraction but differ in the additional reagents and conditions. The wet milling methods discussed in this review are the conventional wet milling, alkali wet milling, enzymatic wet processing, and the modified wet milling method.

Conventional wet milling

In conventional wet milling, dehulled seeds are used as hulls and contain antinutritional constituents that are released during extraction.[11] The dehulled seeds are ground to flour

and treated with a decomposing agent, usually an alkaline solution in order to extract proteins. The protein is then removed by acid precipitation or ultrafiltration. The fiber obtained contains approximately 4–8% protein and 0.5–1.5% lipids.

The conventional wet milling method involves the soaking (steeping) of raw materials in a solution of sulfurous acid. The co-products and starch obtained are then physically separated. Apart from being time- and energy-consuming, this process is also environmentally unfriendly due to the large amounts of sulfur dioxide (SO_2) required during the steeping step. When sulfurous acid reacts with water, SO_2 is produced.[39] In the atmosphere, SO_2 can react with other polluting gases such as nitrogen dioxide (NO_2), forming acid rain. It is also associated with severe respiratory disorders, and it particularly irritates asthmatic individuals.[39] The traditional wet milling steeping process takes up to 36 hours to complete.

Alkali wet milling

The alkali wet milling involves soaking the plant material under study in NaOH (pH 13) at 85 °C.[47] The soaked material is then debranned, cracked, and steeped in NaOH at 45 °C, then ground to a powder. The powder is then blended with the NaOH, and the recovered slurry is degermed, ground, screened, and washed through sieves. The residue is collected as fine fiber.

Enzymatic wet milling

To help curb the problems associated with SO_2, enzymatic wet milling was developed as an alternative.[48] In this process, SO_2 is reduced to minimal levels that only impart antimicrobial properties. The processing time for enzymatic wet milling is reduced and thus saves energy. The enzymes commonly used are protease enzymes such as alcalases, which solubilize and hydrolyze the gluten matrix (protein), heat-stable α-amylase, which gelatinizes, hydrolyses, and depolymerizes starch and amyloglucosidase, which disintegrates starch fragments to glucose.[39] The unchanged nonstarch polysaccharides are recovered by precipitation with ethanol, then washed and dried. Fiber is separated and recovered by eliminating loose starch and proteins by passing over a grit screen.[39]

Modified wet milling

The modified wet milling method is intended for food applications.[35] This method involves the use of water and produces products with a high purity that can be used for a wide range of applications, including scientific research.[38] The first step involves grinding the seeds to very small particles to increase the surface area. The protein is then extracted at an alkaline pH followed by acid precipitation.[35,38] Sodium hydroxide is commonly employed to provide the alkaline pH and HCl for acidic pH.[30]

To isolate insoluble fiber, differences in swelling properties of the fractions are used.[30] At room temperature, fiber has a high swelling capacity whereas the swelling of starch is very restricted. Such differences in swelling capability gives rise to different sizes. The insoluble extract fraction is dispersed in a large amount of water and screened through a series of sieves with pore diameters ranging from 30 to 300 μm.[38] The supernatant is mainly a dispersion of starch granules and the residue is mainly fiber.[35]

In industry, the fibers are dried by spread driers specially constructed for that purpose. For scientific research purposes, freeze-drying of the different fractions is more

appropriate.[38] The modified wet milling method uses much less water than the traditional wet milling method. The modified wet milling only requires the use of chemicals (HCl and NaOH) for pH adjustments in protein precipitation.[30] The purity of leguminous soluble and insoluble fibers extracted using the wet milling method range from 83.3% to 89.6% and from 49.7% to 59.2%, respectively.

Physical and microbial methods

Physical methods of fiber extraction preserve the structure of the fibers and avoid significant damage to the polymer chain. As a result, the extracted fibers tend to have a high cation exchange capacity, as the side chain group remains almost intact.[18] Not much research has been carried out on these methods.

Microbial methods involve the fermentation of fiber using microorganisms and enzymes. Most of the known methods are very specific and precise. Enzymes of high purity are often used to selectively remove oligosaccharides and polysaccharides such as galactans, fructans, mannans, and arabinans.[15] Some of the advantages of microbial isolation are that the structure of the fibers remains undistorted and significant hemicelluloses and soluble fibers are not lost. In addition, the methods have high selectivity and are easy to handle. On the negative side, it is suspected that microbial fermentation produces toxic substances, hence making the extracted fibers unsuitable for use in food applications.[18]

Gravimetric methods

Nonenzymatic-gravimetric methods

Nonenzymatic-gravimetric methods are one of the earliest methods developed for fiber extraction. The methods include hydrolytic or oxidative chemical decomposition, leaving behind crude fiber. These methods can be divided into two categories.[5] The first category includes acid-detergent and neutral-detergent extractions. The acid-detergent procedure isolates crude fiber as the sum of cellulose, lignin, and acid-insoluble hemicelluloses; as a result, most of the fiber components are lost. The neutral-detergent procedure isolates cellulose, lignin, and neutral detergent–insoluble hemicelluloses.[5] It is, however, unsuitable for plants that are high in soluble fiber. The second category makes use of protein and starch-digesting enzymes and is discussed in detail under the enzymatic-gravimetric methods.

Enzymatic-gravimetric methods

The enzymatic-gravimetric method was developed by Prosky et al.[49] based on the work of Asp.[50] This method makes use of enzymatic removal of starch and proteins followed by the precipitation of the soluble fiber concentrate using ethanol.[2] The gravimetric method starts with the use of alkalis and acids to determine crude fiber in plant samples and was later modified by the Association of Analytical Communities (AOAC) to include animal feed. This method was further modified to include the use of enzymes to remove starch and solubilize the protein fraction.[51] The modified method involves the removal of fat if present above 10%.

The enzymatic-gravimetric method has evolved to make use of 4-morpholineethanesulfonic acid–TRIS (MES-TRIS) buffer in place of the original phosphate buffer, thus saving time and

energy associated with the continuous pH adjustments.[5] A modern enzymatic-gravimetric method involves suspending samples in acetate buffer at pH 5, then digesting with heat-stable α-amylase at temperatures between 95 and 100 °C for 30 minutes to 1 hour to digest starch. The samples are further incubated at 60 °C with protease followed by digestion with amyloglucosidase at 60 °C to hydrolyze starch fragments to glucose.[40,41] Soluble fibers are then precipitated with ethanol, and total fiber is recovered by centrifugation with ethanol and acetone and left to dry at room temperature.[41,52]

Fiber was extracted from coconut residue using cold, slightly alkaline water followed by centrifugation at room temperature.[7] The residues were extracted with EDTA.[43] The resultant residues were washed with ethanol and deionized water, then treated with enzymes as discussed in the enzymatic-gravimetric method.[7]

The enzymatic-gravimetric method was applied in extracting fiber from defatted rice bran; however, alcalase was used as the protein-degrading enzyme.[41] The use of pepsin and pancreatin for the digestion of protein and starch is suggested, as these enzymes would mimic the alimentary digestive enzymes.

Enzymatic-chemical methods

The enzymatic-chemical method was first outlined by Southgate in 1969 and developed by Englyst et al. in 1994.[42,53] These methods involve the enzymatic digestion of nonfiber fractions coupled with chemical removal of the fractions. Specifically, the method involves the enzymatic removal of starch and the use of ethanol to isolate the soluble fiber concentrate from products of starch hydrolysis and low-molecular-weight sugars. Ethanol is commonly employed in these methods to precipitate solubilized fiber components as in the enzymatic-gravimetric methods.[24] A crucial step was the removal of all starch to avoid overestimation of total dietary fiber extracted. This was accomplished by use of dimethyl sulfoxide.[5]

The initial step of the American Association for Cereal Chemists (AACC) method involves digestion using H_2SO_4 followed by filtration using water and NaOH. The samples are then washed with H_2SO_4 and ethanol and dried in a muffle oven. The alcoholic fiber extraction method involves boiling material in water for 3 hours and extraction using 95% ethanol, followed by agitation overnight and filtration through a nylon bag using a hydraulic press.[21] The fiber is then recovered by air-drying the residue for 6 hours. Fiber can be subjected to alkali digestion, strained through a cheesecloth, then oven dried overnight.[21] It is recommended to reduce the concentration of ethanol that is used in the precipitation of soluble fiber from 76% to between 41% and 56% to reduce cost and also reduce environmental chemical contamination.[21]

Extraction of individual constituents of fiber

Insoluble pectic substances can be extracted from plant material using heated ammonium oxalate solution followed by filtration, washing with ethanol and distilled water. Hemicelluloses can be extracted from depectinated fiber by centrifugation of the samples with 5% KOH. The residue termed lignocellulose is further centrifuged with 50% acetic acid at pH 5.0–5.5. The residue is dried as hemicellulose. Cellulose can be extracted using $KMNO_4$ mixed with lignin buffer in a ratio 2:1 followed by addition of demineralized solution. Lignin can be extracted using H_2SO_4 at refrigeration temperature. Cold distilled

water is then added, and after precipitation acid is washed off the residue with warm distilled water. The crude lignin can then be air-dried.[7]

Optimization of fiber extraction methods

A method of extracting soluble dietary fiber from wheat, rye, barley, oats, potatoes, carrots, lettuce, and peas was optimized.[52] The study compared four extraction conditions: (1) acetate buffer at pH 5, 96 °C for 1 hour followed by starch degradation at 60 °C for 4 hours; (2) water extraction at 38 °C for 2 hours; (3) HCl/KCl buffer at pH 1.5 at 38 °C for 2 hours; and (4) pretreatment with absolute ethanol at 96 °C for 1 hour followed by water extraction at 38 °C for 2 hours. High-temperature extraction gave the highest extraction yield, whereas acidic extraction gave the least. Carrots yielded the highest total dietary fiber (TDF) and potatoes yielded the least. The researchers concluded that extraction conditions affect the yield and the composition of the resultant fibers.

The extraction of dietary fiber from date seeds was optimized. The seeds were dried at 50 °C for 2 days, ground, and then fiber was extracted using water on some samples and acetone on others.[37] After stirring for 1 hour at 40 °C, centrifugation, and filtration, butanone and butanol were employed for purification of the fibers. The residue was dried at 60 °C and ground as dietary fiber concentrate. The researchers concluded that the use of water extraction followed by purification using butanol gives the highest yield as compared with water-butanone, acetone-butanone, and acetone-butanol. The researchers chose butanol and butanone for purification because of their low toxicity levels, economy due to low evaporation temperature, and high affinity to dissolve compounds with hydroxyl groups. The yield of total dietary fiber from the date seeds was 93.5% (water-butanol), 91.2% (water-butanone), 88.8% (acetone-butanol), and 81.9% (acetone-butanone). Acetone was found to extract a higher amount of phenolics with the fiber because of its high extraction efficiency.

The extraction of soluble dietary fiber from defatted rice bran was optimized using response surface methodology.[54] The independent variables they investigated were the ratio of $Ca(OH)_2$ solution to defatted rice bran, concentration of $Ca(OH)_2$, and extraction temperature, with optimal values of 29.75:1 (mL/g), 3%, and 84 °C (1 hour), respectively. The method involved digesting defatted rice bran with α-amylase at 90 °C and pH 6.9 for 15 minutes. After digestion the mixture was filtered, washed with deionized water, and mixed with $Ca(OH)_2$ for 4 hours. The alkali was then neutralized with acetic acid, centrifuged, dialyzed against deionized water, and precipitated with 80% ethanol. The yield of fiber was 7.86 g/100 g.

Fiber extraction from Maixiansan was optimized using response surface methodology.[55] The procedure involved soaking Maixiansan in boiled water, digestion of starch with α-amylase until the enzyme was inactivated, washing, centrifugation, and drying in a rotary evaporator. After optimization, the highest fiber yield of 57.14% was obtained using an enzyme concentration of 0.4%, 45 minutes enzymolysis time, and 4% NaOH content.

Soluble fiber was extracted from defatted rice bran using (1) NaOH pH 14, (2) CaOH pH 12, (3) $NaCO_3$ pH 11, (4) acetic acid pH 3, and (5) HCl pH 0.5.[57] All the treatments involved the predigestion of starch using glucoamylase, blending sample with the appropriate reagent followed by shaking at 60 °C for 4 hours, centrifugation, and neutralizing

with acetic acid. Some samples were treated with trichloroacetic acid. The samples then underwent dialysis under tap water for 3 days, then soaked in 95% ethanol overnight, centrifuged, then redissolved in water and freeze-dried. The yield of fiber was 8%, 5%, 2%, 2%, and 4% for each treatment, respectively. Trichloroacetic acid was applied for protein removal; however, it was noted that it was inefficient, as there was no significance difference in the protein content of treated and untreated samples. It was observed that even though extraction with NaOH gave the highest yield, it was inappropriate for food applications, as it imparted a strong brown color to the fibers. Treatment with CaOH would be more appropriate because it gave the least discoloration, had a desirable composition of fibers, and had a satisfactory yield. In addition, the fibers treated with CaOH retained their hypocholesterolemic activity.

Comparison of fiber extraction methods

Dry processing methods are suitable for plants that have starch as their main storage material. These methods require repeated classification to purify fractions, and this is a drawback because it reduces product recovery.[38] The conventional wet milling process makes use of large amounts of sulfur dioxide (SO_2) during the steeping step. SO_2 is environmentally unfriendly, as it reacts with other polluting gases such as nitrogen dioxide (NO_2) in the atmosphere, forming acid rain. It is also associated with severe respiratory disorders, and it particularly irritates asthmatic individuals.[39] The conventional wet milling steeping process is very time-consuming, taking up to 36 hours to complete. In the conventional wet milling method, steeping and evaporation of steep water is estimated to use up to 21% of the total capital and energy, hence making it very costly.[47]

The alkali wet milling method is equally tedious and time-consuming as the conventional wet milling method. The process, however, produces a relatively environmentally acceptable stream of waste water.[47]

The enzymatic wet milling method was developed to overcome the problems associated with the conventional wet milling process. In this method, SO_2 is reduced to minimal levels to impart antimicrobial properties and the processing time is also reduced.[39,49]

The modified wet milling method involves the use of water and produces products with a high purity that can be used for a wide range of applications, including scientific research.[38] The modified wet milling method uses much less water than the traditional wet milling method and does not require the use of any chemical.[30]

Enzymatic-gravimetric methods extract a group of polysaccharides, lignin, some resistant starch, waxes, Maillard reaction products, and phenolic compounds. They give a higher yield of fiber of almost 2-fold compared with enzymatic-chemical methods.[40,58] They are also quick, easier to carry out, and do not overestimate fiber.[2] The limitation of these methods is that some insoluble polysaccharides, lignin, and all soluble polysaccharides are lost. The residue obtained also contains some nitrogenous material and oligosaccharides, and some resistant starch are not quantified.[5,58] Dialysis, which is employed in the purification of soluble fiber, is costly.

The enzymatic-chemical method is faster and easier to perform relative to nonenzymatic-gravimetric methods.[59] However, it is environmentally unfriendly due to the use of various chemicals, which if not correctly handled or disposed of pose a threat to

Table 4. Advantages and limitations of fiber extraction methods.

Method	Advantages	Limitations	Reference
Dry processing	Reduced water and energy consumption No reagents used	Only for plants with starch as main storage Low yield	(11, 39)
Conventional wet milling	Appreciable amount of fiber obtained	Large amounts of SO_2 Time-consuming Very costly	(40)
Alkali wet milling	Less waste water produced	Tedious Time-consuming	(48)
Enzymatic wet milling	SO_2 reduced to a minimum Processing time reduced	Possible SO_2 residues in product	(40)
Modified wet milling	High-purity products Much less water used No chemicals	Waste water	(31)
Enzymatic-gravimetric	Higher yield than enzymatic-chemical Quick and easy to carry out	Some insoluble fibers, lignin, and all soluble fibers are lost Residues contain nitrogenous material	(58)
Enzymatic-chemical	Fast and easy to perform compared with enzymatic-gravimetric	Chemical residues in products Time-consuming	(2, 38)
Nonenzymatic-gravimetric	High-purity fibers	Poor selectivity Extractions conditions difficult to control	(19)
Physical	Structure of the fibers preserved	Unreliable	(19)
Microbial	Structure of fibers maintained High selectivity Easy to handle	Toxic substances produced	(19)

the environment.[37] These methods are also tedious and time-consuming, and chemicals are also of concern due to the possibility of solvent residues in the product.[2,37]

Nonenzymatic-gravimetric methods also make use of chemicals and isolate cellulose, lignin, and acid-insoluble hemicelluloses. The use of chemicals improves the removal of starch and proteins, thus providing a fiber of high purity. However, chemical methods have poor selectivity and the extraction conditions are difficult to control, thus limiting their use.[18] In addition, alkaline solutions dissolve hemicelluloses and some soluble fibers, therefore rendering them unavailable in the extracted fiber.[41]

An ideal method of extraction ought to be environmentally friendly, safe, easy to perform, and cost-effective. Chemicals, enzymes, and equipments used in many of the methods tend to be very costly. The wet milling method makes use of water and minimal chemicals and thus is concluded to be one of the most cost-effective methods of fiber extraction. Table 4 gives the advantages and disadvantages of the methods discussed in this review. Knowledge of the advantages and limitations of extraction methods is of importance when selecting a method for extracting a particular type of fiber from a certain plant. A method that is less costly and uses minimal chemicals is considered preferable.

Table 5 gives a comparison of some fiber extraction methods developed by different scholars over the years. With advances in technology and an increase in the knowledge pool, more effective, robust, and reproducible fiber extraction methods have been developed.[60] However, the work done on fiber extraction methods is still very limited and more research is required.

Table 5. Different fiber extraction methods and their applications.

Method	Products	Reference
Enzymatic-colorimetric	Cellulose and lignin	(43)
Wet processing	Soluble fiber	(44)
Enzymatic-gravimetric	Insoluble and soluble fibers	(51)
Enzymatic-chemical	Hemicellulose, cellulose, lignin	(45)
Enzymatic-gravimetric	Crude fiber	(50)
Chemical	Soluble fiber	(57)
Enzymatic-chemical	Total dietary fiber	(54)
Gravimetric	Hemicellulose, cellulose, lignin	(46)
Enzymatic-chemical	Hemicellulose, cellulose, lignin	(47)
Chemical and wet processing	Soluble fiber	(53)
Enzymatic-chemical	Soluble fiber	(55)
Chemical	Total dietary fiber	(22)
Modified wet milling	Insoluble and soluble fibers	(31)
Chemical and wet processing	Total dietary fiber	(38)
Enzymatic-gravimetric	Total dietary fiber	(7)
Enzymatic-gravimetric	Total dietary fiber	(42)

Effects of extraction on the characteristics of fibers

Different extraction conditions affect the characteristics of the resultant fibers. Enzymatic digestion and alkaline extraction partially delignifies lignocelluloses.[5] This results in improved fiber functionality in food products. Enzymes also modify the soluble fiber to insoluble fiber ratio; for example, xylanase raises the level of soluble dietary fibers when applied to cell walls. Mechanical applications such as grinding prior to extraction disrupt the fiber network, exposing hydroxyl groups, thus increasing the water holding capacity of fiber and increase the surface area, hence increasing the fiber yield after extraction. However, excessive grinding results in reduced water-holding capacity due to extensively disrupted fiber. Mechanical applications also affect the particle size of the resultant fibers. The smaller the particle size, the higher the fat binding capacity.

Physical extraction methods such as extrusion result in an increase in total dietary fiber and an overall decrease in lignin content. In addition, the process increases the soluble to insoluble fiber ratio and also increases the water holding capacity.[2] Soaking, a common stage in the preparation of plant material prior to extraction, modifies the composition and availability of fibers. Heat changes the soluble to insoluble fiber ratio and alters the total dietary fiber content. The increase in fiber content is attributed to the formation of fiber-protein complexes that are heat resistant and are collected as fiber.

Relevance of fiber for the food industry

The physical and chemical properties of fiber are of importance in predicting its functional behavior in food systems.[11] These properties include solubility, fat binding capacity, water holding capacity, density, swelling capacity, gel-forming ability, mineral and organic molecule binding capacity, flavor, color, and rheological properties.[11,17]

Many fiber-deficient food systems are fortified with dietary fiber from several sources to help improve their nutritional value. The majority of foods consumed on a daily basis have low DF content, containing approximately 1–3% fiber. A higher amount of DF is found in less popular foods such as whole grain cereals, legumes, and dried fruits.[10]

Table 6. Various properties of fiber that can be used in food products.

Industry	Product	Property of fiber
Meat, fish	Sausages, polony, bologna, burgers	Fat replacer, emulsion stabilizer, water binder, reduce lipid oxidation, improve cooking yield, improve texture
Bakery	Bread, baked products	Modify texture, increase volume, increase shelf life, modify bread volume, improve firmness of loaf, modify springiness, increase softness of the crumb, replace wheat flour, improve nutritional quality
Dairy	Ice cream, yogurts, cheese	Improve body, reduce syneresis, improve mouthfeel
Beverage	Juice, drinks	Bulking agent, nutritional additive, improve viscosity and stability
Sauce	Sauces	Thickener
Breakfast cereals	Breakfast cereals	Fortification—improved fiber content
Confectionery	Sweets, chocolates	Sugar substitutes, reduce calorie content
Fast food	Fried products	Reduce oil retention
Extruded products	Pasta	Fortifying agent
Fruit products	Jam, marmalade	Improve pseudoplastic behavior, stability, increased shelf life

Note. Adapted from references 5, 11, 19, 42, and 62.

The food industry may use the properties of fiber to their advantage by incorporating it in various food products.[5,62] Table 6 shows the various industries that stand to benefit from the increased use of fibers from various sources. Fiber can alter the consistency, rheological behavior, texture, and sensory properties of foods.[11,41] Functionally, fibers can be employed as bulking agents in reduced sugar applications, water binders in reduced fat applications, impart antioxidant properties, and contribute to sensory characteristics.[34]

Fibers can also be applied to improve the shelf life of foodstuffs due to their gel-forming, anticlumping, antistick, fat mimetic, thickening, and water holding capabilities.[18] They may also be used as replacements to additives, thus offering various products a "clean label." Dietary fiber may be included in diets of groups that are prone to fiber deficiency, such as dysphagic groups who might otherwise have low fiber intake.[41] Several types of fibers for use in food products are given in the literature. These include citrus fiber, pineapple fiber, fractions of grains and multi-fruits, pectins, β-glucans, cellulose beet-root fiber, polydextrose, oat bran, potato peel fiber, legume fiber, defatted rice bran, and date fiber.[16,18]

A fiber source is chosen based on its nutritional quality, amounts of total and soluble fiber, caloric content, antioxidant capacity, grade of fermentability, and water retention.[15,18] In order for a particular fiber to be an acceptable food ingredient, it must, among others, have a good shelf life, a high concentration in small quantities so as to maximize its use, a balanced composition of soluble and insoluble fibers, as well as be compatible with food processing. Furthermore, the fiber must have a bland taste and not have antinutritional components, an offensive odor, or negative color and textural effects. The fiber must also have an adequate amount of associated bioactive compounds, be of reasonable cost, and have a positive consumer image.[6,19,62,63]

References

1. Chau, C.F.; Huang, Y.L. Characterization of passion fruit seed fibres—A potential fibre source. Food Chem. **2004**, *85*, 189–194.

2. Dhingra, D.; Michael, M.; Rajput, H.; Patil, R.T. Dietary fibre in foods: A review. J. Food Sci. Technol. **2012**, *49*, 255–266.
3. Khan, A.R.; Alam, S.; Ali, S.; Bibi, S.; Khalil, I.A. Dietary fiber profile of food legumes. Sarhad J. Agric. **2007**, *23*, 763–766.
4. Rosell, C.M.; Santos, E.; Collar, C. Physico-chemical properties of commercial fibres from different sources: A comparative approach. Food Res. Int. **2009**, *42*, 176–184.
5. Elleuch, M.; Bedigian, D.; Roiseux, O.; Besbes, S; Blecker, C. Dietary fibre and fibre-rich by-products of food processing; Characterisation technological functionality and commercial applications: A review. Food Chem. **2010**, *124*, 411–421.
6. Figuerola, F.; Hurtado, M.L.; Estevez, A.M.; Chiffelle, I.; Asenjo, F. Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chem. **2005**, *91*, 395–401.
7. Verma, A.K.; Banerjee, R. Dietary fibre as functional ingredient in meat products: A novel approach for healthy living—A review. J. Food Sci. Technol. **2010**, *47*, 247–257.
8. Westenbrink, S.; Brunt, K.; Van de Kamp, J.W. Dietary fibre: Challenges in production and use of food composition data. Food Chem. **2012**, *140*, 562–567.
9. Slavin, J. Fiber and prebiotics: Mechanisms and health benefits. Nutrients **2013**, *5*, 1417–1435.
10. Guillon, F.; Champ, M.M. Carbohydrate fractions of legumes: Uses in human nutrition and potential for health. Br. J. Nutr. **2000**, *88*, 293–306.
11. American Association of Cereal Chemists. The definition of dietary fiber: American Association of Cereal Chemists. Cereal Food. World. **2001**, *46*, 112–126.
12. Borderias, A.J.; Sanchez-Alonso, I.; Perez-Mateos, M. New applications of fibres in foods: Addition to fishery products. Trends Food Sci. Technol. **2005**, *16*, 458–465.
13. Garcia, M.L.; Caceres, E.; Selgas, M.D. Utilisation of fruit fibres in conventional and reduced-fat cooked-meat sausages. J. Sci. Food Agric. **2007**, *87*, 624–631.
14. Codex Alimentarius Commission. *Nutrition and Foods for Special Dietary Uses*; Report on the 30th session of the Codex Committee; Codex Alimentarius Commission: Rome, **2009**.
15. Rodriguez, R.A.J.; Fernandez-Bolanos J.; Guillen R.; Heredia A. Dietary fibre from vegetable products as a source of functional ingredients. Trends Food Sci. Technol. **2006**, *17*, 3–15.
16. Huang, Y.L.; Chow, C.J.; Fang, Y.J. Preparation and physicochemical properties of fiber-rich fraction from pineapple peels as a potential ingredient. J. Food Drug Anal. **2011**, *19*, 318–323.
17. Biswas, A.K.; Kumar, V.; Bhosle, S.; Sahoo, J.; Chatli, M.K. Dietary fibers as functional ingredients in meat products and their role in human health. Int. J. Livest. Prod. **2011**, *2*, 45–54.
18. Yangilar, F. The application of dietary fibre in food industry: Structural features, effects on health and definition, obtaining and analysis of dietary fibre: A review. J. Food Nutri. Res. **2013**, *3*, 13–23.
19. Mwaikambo, L.Y. Review of the history, properties and application of plant fibres. Afr. J. Sci. Technol. **2006**, *7*, 120–133.
20. Trumbo, P.; Schlicker, S.; Yates, A.A.; Poos, M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J. Am. Diet. Assoc. **2003**, *103*, 563.
21. Prakongpan, T.; Nitithamyong, A.; Luangpituksa, P. Extraction and application of dietary fiber and cellulose from pineapple cores. J. Food Sci. **2002**, *67*, 1308–1313.
22. Esposito, F.; Arlotti, G.; Bonifati, A.M.; Napolitano, A.; Vitale, D.; Fogliano, V. Antioxidant activity and dietary fibre in durum wheat bran by-products. Food Res. Int. **2005**, *38*, 1167–1173.
23. Jenkins, D.J.A.; Kendall, C.W.C.; Vuksan, V.; Vidgen, E.; Parler, T.; Faulkner, D.; Mehling, C.C.; Garsetti, M.; Testolin, G.; Cunnane, S.C.; Ryan, M.A.; Corey P.N. Soluble fiber intake at a dose approved by the US Food and Drug Administration for a health claim of health benefits: Serum lipid risk factors for cardiovascular disease assessed in a randomized controlled crossover trial. Am. J. Clin. Nutr. **2002**, *75*, 834–839.
24. Diedericks, C. Functional properties of Bambara groundnut (*Vigna Subterranea* (L) Verdc) non-starch polysaccharides in model and food systems. Master's thesis; Cape Peninsula University of Technology: Cape Town, South Africa, **2014**.

25. Wood, J.A.; Grusak, M.A. *Nutritional Value of Chickpea*; Cromwell Press: Trowbridge, UK, **2007**.
26. Hawkes C. Uneven dietary development: Linking the policies of globalization with the nutrition transition, obesity, and diet-related chronic diseases. Global Health **2006**, *2*, 4.
27. Fernandez-Lopez, J.; Fernandez-Gines, J.M.; Aleson-Carbonell, L.; Sendra, E.; Sayas-Barbera, E.; Perez-Alvarez, J.A. Application of functional citrus by-products to meat products. Trends Food Sci. Technol. **2004**, *15*, 176–185.
28. Cava, R.; Ladero, L.; Cantero, V.; Ramirez, M.R. Assessment of different dietary fibers (tomato fiber, beet root fiber, and inulin) for the manufacture of chopped cooked chicken products. J. Food Sci. **2012**, *77*, 346–352.
29. Choi, Y.S.; Kim, H.W.; Hwang, K.E.; Song, D.H.; Choi, J.H.; Lee, M.A.; Chunh, H.J.; Kim, C.J. Physicochemical properties and sensory characteristics of reduced-fat frankfurters with pork back fat replaced by dietary fiber extracted from makgeolli lees. Meat Sci. **2014**, *96*, 892–900.
30. Dalgetty, D.D.; Baik, B. Isolation and characterisation of cotyledon fibers from peas, lentils, and chickpeas. AACC **2003**, *80*, 310–315.
31. Ojimelukwe, P.C. *Sourcing and Processing of Legumes*; Raw Materials Research and Development Council: Abuja, Nigeria, **2009**.
32. Fasoyiro, S.B.; Ajibade, S.R.; Omole, A.J.; Adeniyan, O.N.; Farinde, E.O. Proximate, mineral and anti-nutritional factors of some underutilized grain legumes in South-Western Nigeria. Nutr. Food Sci. **2006**, *38*, 18–23.
33. Murevanhema, Y.Y.; Jideani, V.A. Potential of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk as a probiotic beverage: A review. Crit. Rev. Food Sci. Nutr. **2013**, *53*, 954–967.
34. Ramirez-Santiago C.; Ramos-Solis, L.; Lobato-Calleros, C.; Pena-Valdivia, C.; Vernon-Carter, E. J.; Alvarez-Ramirez, J. Enrichment of stirred yogurt with soluble dietary fiber from *Pachyrhizus erosus* L. Urban: Effect on syneresis, microstructure and rheological properties. J. Food Eng. **2010**, *101*, 229–235.
35. Anonymous. *Processing Methods For Dry Peas, Lentils & Chickpeas*; Dry Pea & Lentil Council: Moscow, ID, **2010**.
36. Fuentes-Alventosa, J.M.; Rodriguez-Gutierrez, G.; Jaramillo-Carmona, S.; Espejo-Calvo, J.A.; Rodriguez-Arcos, R.; Fernandez-Bolanos, J.; Guillen-Bejarano, R.; Jimenez-Araujo, A. Effect of extraction method on chemical composition and functional characteristics of high dietary fibre powders obtained from asparagus by-products. Food Chem. **2009**, *113*, 665–671.
37. Al-Farsi, M.A.; Lee, C.Y. Optimization of phenolics and dietary fibre extraction from date seeds. Food Chem. **2008**, *108*, 977–985.
38. Bogracheva, T.; Cserhalmi, Z.; Czukor, B.; Fornal, J.; Schuster-Gajzago, I.; Kovacs, E.T.; Lewandowicz, G.; Soral-Smietana, M. xxx. In *Carbohydrates in Grain Legume Seeds: Improving Nutritional Quality and Agronomics Characteristics*; CABI Publishing: New York: **2001**; pp 89–92.
39. Ramirez, E.A.; Johnston, D.B.; McAloon, A.J.; Singh, V. Enzymatic corn wet milling: Engineering process and cost model. Biotechnol. Biofuel. **2009**, *2*, 2.
40. Salehifar M.; Fadaei V. Comparison of some functional properties and chemical constituents of dietary fibers of Iranian rice bran extracted by chemical and enzymatic methods. Afr. J. Biotechnol. **2011**, *8*, 18525–18531.
41. Daou, C.; Zhang, H. Study on functional properties of physically modified dietary fibres derived from defatted rice bran. J. Agric. Sci. **2012**, *4*, 85–97.
42. Southgate, D.A.T. Determination of carbohydrates in foods: Unavailable carbohydrates. J. Sci. Food Agric. **1969**, *20*, 331–335.
43. Furda. I. Fractionation and examination of biopolymers from dietary fibre. Cereal Food World **1977**, *22*, 252–254.
44. Anderson, N.E.; Clydesdale, F.M. An analysis of the dietary fiber content of a standard wheat bran. J. Food Sci. **1980**, *45*, 336–340.
45. Lawther, J.M.; Sun, R.; Banks, W.B. Extraction, fractionation, and characterization of structural polysaccharides from wheat straw. J. Agric Food Chem. **1995**, *43*, 667–675.

46. Claye, S. S.; Idouraine, A.; Weber, C.W. Dietary fibre, what it is and how it is measured. Food Chem. **1996**, *57*, 305–310.
47. Eckhoff, S.R.; Du, L.; Yang, P.; Rausch, K.D.; Wang, D.L.; Li, B.H.; Tumbleson, M.E. Comparison between alkali and conventional corn wet-milling: 100-g procedures. Cereal Chem. **1999**, *76*, 96–99.
48. American Association for Cereal Chemists. *Chemistry and Technology*; American Association for Cereal Chemists: St. Paul, MN, 1987.
49. Prosky, L.; Asp, N.G.; Scheweizer, T.F.; DeVries, J.W.; Furda, I. Determination of insoluble and soluble, and total dietary fibre in foods and food products: Interlaboratory study. J. AOAC **1988**, *71*, 1017–1023.
50. Asp, N.G. Critical evaluation of some suggested methods for assay of dietary fibre. J. Plant Technol. **1978**, *3*, 21–25.
51. Association of Analytical Communities. *Method 991.43 for Soluble and Insoluble Dietary Fibre*; Association of Analytical Communities: Gaithersburg, MD, **2000**.
52. Graham, H.; Rydberg, M.B.G.; Aman, P. Extraction of soluble dietary fibre. J. Agric. Food Chem. **1988**, *36*, 494–497.
53. Englyst, H.N.; Quigley, M.E.; Hudson, G.J. Determination of dietary fiber as non-starch polysaccharides with gas-liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst **1994**, *119*, 1497–1509.
54. Wan, Y.; Espinoza Rodezno, L.A.; Mis Solval, K.; Li, J.; Sathivel, S. Optimization of soluble dietary fiber extraction from defatted rice bran using response surface methodology. J. Food Process. Preserv. **2002**, *38*, 441–448.
55. Lv, H.; Hu, C.; Zhong, H.; Zheng, H.; Wen, C. Optimization of technology for dietary fiber extraction from Maixiansan by response surface methodology. Chin. Med. **2010**, *7*, 28.
56. Aoe, S.; Oda, T.; Tatsumi, K.; Yamauchi, M.; Ayano, Y. Extraction of soluble dietary fibre from defatted rice bran. Cereal Chem. **1993**, *70*, 423–425.
57. Fadaei, V.; Salehifar, M. Some chemical and functional characteristics of dietary fiber from five fiber sources. Eur. J. Exp. Biol. **2012**, *2*, 525–528.
58. Gordon, D. T.; Okuma, K. Determination of total dietary fiber in selected foods containing resistant maltodextrin by enzymatic-gravimetric method and liquid chromatography: Collaborative study. J AOAC Int. **2002**, *85*, 435–444.
59. Caprita, R.; Caprita, A. Chemical methods for the determination of soluble and insoluble non-starch polysaccharides—Review. J Anim. Sci Biotechnol. **2011**, *44*, 73–80.
60. Bach Knudsen, K.E. The nutritional significance of "dietary fiber" analysis, Anim. Feed Sci. Technol. **2001**, *90*, 3–20.
61. Tomaschunas, M.; Zorb, R.; Fischer, J.; Kohn, E.; Hinrichs, J.; Busch-Stockfisch, M. Changes in sensory properties and consumer acceptance of reduced fat pork Lyon-style and liver sausages containing inulin and citrus fiber as fat replacers. Meat Sci. **2013**, *95*, 629–640.
62. Kunzek, H.; Müller, S.; Vetter, S.; Godeck, R. The significance of physicochemical properties of plant cell wall materials for the development of innovative food products. Euro. Food Res. Technol. **2002**, *214*, 361–376.
63. Dhepe, P.L.; Sahu, R. A solid-acid-based process for the conversion of hemicelluloses. Green Chem. **2010**, *12*, 2153–2156.
64. Vanholme, R; Demedts, B; Morreel, K.; Ralph, J; Boerjan, W. Lignin biosynthesis and structure. Plant Physiol. **2010**, *153*, 895–905.
65. Coultate, T.P. Food*: The Chemistry of Its Components*; The Royal Society of Chemistry: Cambridge, UK, **2009**.
66. Gropper, S.S; Smith, J.L; Groff, J.L. xxx. In *Advanced Nutrition and Human Metabolism*. Wadsworth: Belmont, CA, **2009**; pp 166–121.
67. Ophardt, C.E. Carbohydrates (cellulose). Chem. Environ. Issues **2003**, *103*, xx–xx.

Appendix B: Peer reviewed publication in the *South African Journal of Science* titled "Physicochemical characteristics of Bambara Groundnut dietary fibres extracted using wet milling.

Physicochemical characteristics of Bambara groundnut dietary fibres extracted using wet milling

AUTHORS:
Yvonne Maphosa[1]
Victoria. A. Jideani[1]

AFFILIATION:
[1]Department of Food Technology, Cape Peninsula University of Technology, Cape Town, South Africa

CORRESPONDENCE TO:
Yvonne Maphosa

EMAIL:
yvonmaphosa@gmail.com

POSTAL ADDRESS:
Department of Food Technology, Cape Peninsula University of Technology, PO Box 1906, Bellville 7535, South Africa

DATES:
Received: 30 Mar. 2015
Revised: 17 May 2015
Accepted: 06 July 2015

KEYWORDS:
swelling capacity; bulk density; water holding capacity; soluble dietary fibre; insoluble dietary fibre

HOW TO CITE:
Maphosa Y, Jideani VA. Physicochemical characteristics of Bambara groundnut dietary fibres extracted using wet milling. S Afr J Sci. 2016;112(1/2), Art. #2015-0126, 8 pages. http://dx.doi.org/10.17159/sajs.2016/20150126

The objectives of this study were to extract soluble and insoluble dietary fibres from four Bambara groundnut (BGN) varieties (black-eye, brown-eye, brown and red) using the wet milling method and evaluate their physicochemical properties. The swelling capacities of brown-eye (6.5 g/mL) and black-eye (6.2 g/mL) fibres were higher ($p \leq 0.05$) than those of red (6.0 g/mL) and brown (5.5 g/mL) fibres while the water holding capacities of black-eye and brown-eye fibres (2.84 g and 2.83 g water/g sample) were higher ($p \leq 0.05$) than those of brown and red fibres. The bulk densities of insoluble dietary fibres (IDFs) and soluble dietary fibres (SDFs) ranged between 0.57 g/mL (red) to 0.67 g/mL (brown-eye) and 0.46 g/mL (brown-eye) to 0.57 g/mL (black-eye), respectively. The oil binding capacities (OBCs) of SDFs ranged between 2.78 g oil/g sample (brown) and 4.03 g oil/g sample (brown-eye) while the OBC of all IDFs did not differ ($p > 0.05$), ranging between 1.52 g oil/g sample (brown) and 1.40 g oil/g sample (brown-eye and black-eye). Black-eye and brown-eye dietary fibres had higher phenolic and total sugar content. The findings of this study indicate the potential of BGN fibres in food systems as fat replacers, emulsion stabilisers, water binders, bulking agents, thickeners and nutritional additives.

Introduction

Bambara groundnut (BGN) is an underutilised crop predominantly grown in African countries.[1,2] Legume seeds such as BGN are good sources of dietary fibre[3] and BGN fibre has potential for both food and non-food applications.[4] An increase in consumer awareness of the health benefits of dietary fibre (DF) has led to the investigation of alternative sources by a number of researchers.[5-7] These health benefits include reduced risk of diseases of lifestyle, such as obesity, diabetes, coronary heart disease, some cancers and haemorrhoids.[7-9]

Legumes that have been researched for DF extraction include cowpeas, lentils and chickpeas.[5,10] The basis of DF extraction methods is similar, however, the approach differs depending on the desired end product, source of fibre and availability of equipment. All DF extraction methods involve fractionation as this allows for the separation of constituents to obtain the desired concentrates and isolates.[11] Some methods of extracting DF include microbiological retting, chemical, enzymatic, dry processing and wet processing.[12]

The modified wet milling method as reported by Dalgetty and Baik[5] is more efficient than the conventional wet methods that rely solely on the differences in swelling capacity to separate starch and fibres, as it makes use of the enzyme α-amylase to digest any remaining starch, thus purifying the fibre concentrate. Extracted DF from BGN using the wet milling method is not documented. Furthermore, the properties and applications of BGN DFs are not largely documented. An understanding of the physicochemical properties of BGN DF will highlight the behaviour in different food and non-food systems including in the human gastro-intestinal tract.[13,14] Diedericks[15] applied an enzymatic-gravimetric method of extracting DF from BGN. The method proved to be very costly and time consuming costing approximately ZAR26 388.57/kg DF. The wet milling method is cheaper and easy to handle.[5] Therefore, the objectives of this study were to extract soluble and insoluble fibres from whole seeds of BGN varieties using the wet milling method as an alternative to the enzymatic-gravimetric method and evaluate their physicochemical properties.

Materials and Methods

Materials

BGN seeds were purchased from Triotrade in Johannesburg, South Africa, and sorted into four varieties according to the 'eye' colour, namely, the black-eye, brown-eye, brown and red varieties. Chemicals used in this study were of analytical grade (Sigma-Aldrich, Johannesburg, South Africa). Equipment used was obtained from the Departments of Food Technology and Oxidative Stress of the Cape Peninsula University of Technology.

Milling of Bambara groundnut seeds

BGN seeds were washed and then dried at 50 °C for 48 h (Cabinet drier, Model: 1069616, Geiger & Klotzbucher, Cape Town, South Africa). The seeds were then milled using a hammer mill (Bauermeister, Bauermeister Inc., Vernon Hills, IL, USA) with a sieve size of 250 μm.

Wet fractionation of BGN flour into individual constituents

The method of Dalgetty and Baik[5] was adopted in this study. BGN flour (200 g) was mixed with 500 mL distilled water and blended for 3 min at the highest setting. The slurry was centrifuged (15 min, 25 °C, 1500 x *g*). The residue was used for the isolation of insoluble dietary fibre (IDF) and the supernatant was used in the isolation of soluble dietary fibre (SDF).

Isolation of BGN insoluble dietary fibre

The residue (26 g) was wet screened in 2 L of water through a 53 μm sieve. The supernatant was collected as starch concentrate. To purify IDF, the collected sediment was digested using 13 units/mg 10 MU heat-stable α-amylase in 400 mL of water at pH 6 for 30 min, in a shaking water bath (100 °C). After digestion, the tubes were left to cool down to room temperature and centrifuged (10 min, 25 °C, 1500 x *g*). The residue was collected and dried at 50 °C (Cabinet drier, Model: 1069616) for 48 h and then vacuum dried in an air oven at 100 °C for 2.5 h.

Isolation of soluble dietary fibre

Soluble dietary fibre was isolated from the supernatant collected after wet fractionation. Firstly, proteins were precipitated by adjusting the pH of the soluble fraction from pH 3 to pH 9 using 1 N NaOH and 1 N HCl. Following precipitation, the soluble fraction was centrifuged (10 min, 25 °C, 1500 x *g*). The sediment was collected as protein concentrate. The supernatant was subjected to a tangential flow filtration system (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) and each fibre solution was washed with four diafiltration volumes to remove any contaminants. Waste was removed through a hollow fibre filtration outlet with a molecular weight cut-off of 10 kD.

Assessment of the physicochemical properties of BGN fibres

Hydration properties

The swelling capacities of BGN IDFs were determined using the method of Wang and Toews.[16] Dry, purified IDF (0.2 g) was hydrated with 10 mL of distilled water in a graduated cylinder and left to stand at 24 °C for 18 h. The swelling capacity of the fibres was then calculated as the bed volume occupied by fibres per gram of dry sample.

The method described by Dalgetty and Baik[5] was applied with modifications in the determination of the water holding capacity (WHC) of BGN IDFs. In a 50 mL centrifuge tube, 1 g of fibre and 30 mL of distilled water were added and the tubes were held for 18 h at 24 °C to allow sufficient hydration of the fibre. The tubes were then centrifuged (3000 x *g*, 20 min, 23 °C). The supernatant was decanted and the tubes carefully inverted for 10 min to drain any remaining free water. The weight of the residue was then recorded and the difference between the original volume of water and the volume of the supernatant was calculated to determine the WHC. The WHC was expressed as mL/g.

Density of BGN fibres

The method of Parrott and Thrall[17] was followed in determining both bulk and direct densities. Bulk density was determined by adding 2 g of each BGN fibre into a graduated syringe and manually applying sufficient pressure while gently tapping the syringe on a bench until the contents were packed tightly. Direct density was determined by adding fibre to the 5 mL mark in a 10 mL graduated cylinder. Care was taken to avoid shaking the cylinder so as to avoid settling of the fibre.[5] The dietary fibre was then emptied and weighed. Bulk and direct densities were expressed in g/mL.

Oil binding capacity of BGN fibres

The method described by Dalgetty and Baik[5] was applied to determine the oil binding capacity (OBC) of the BGN fibres with modifications. Fibre (1 g) was mixed with 5 g of canola oil in a 50 mL centrifuge tube. The mixture was vortexed for 30 sec at 5 min intervals for 30 min. The mixture was then centrifuged (1600 x *g*, 25 min, 23 °C). After centrifugation, the supernatant (free oil) was decanted and weighed. OBC was expressed as grams of oil retained/grams of fibre.

Colour measurements of BGN fibres

Colour attributes of BGN dietary fibres were determined using a spectrophotometer (Model CM-5, Konica Minolta Sensing, Osaka, Japan) set at standard observer 10° and D65. The spectrophotometer was calibrated with black and white plates followed by zero calibration. BGN fibres (3 g of IDF and 0.6–0.8 g SDF) were placed in a glass sample holder (diameter 30 mm). Lightness (L*), redness/greenness (a*) and yellowness/blueness (b*), hue and chromacity were assessed through L*C*h* and CIE-L*a*b* colour space systems. Each variety was analysed in triplicate with each individual sample giving three readings. Colour differences amongst the fibre samples were calculated using the colour difference equation:

$$\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})} \qquad \text{Equation 1}$$

where L* is lightness, a* is redness/greenness and b* is yellowness/blueness

Assessment of polyphenolic compounds in BGN fibres

The method of Diedericks[15] was adopted in the assessment of polyphenolic compounds. Condensed tannins were determined in IDFs while hydrolysable polyphenols (HPPs) were determined in both SDFs and IDFs. For determination of HPPs in BGN IDFs, samples (250 mg) were mixed with 10 mL of methanol and 1 mL of H_2SO_4 in 14 mL centrifuge tubes. The samples were incubated at 80 °C for 20 h. The samples were then centrifuged (4000 x *g,* 5 min, 21 °C) and the residues were analysed using the Folin–Ciocalteu assay by mixing 25 μL of sample with 125 μL of 0.2 M Folin–Ciocalteu and 100 μL of 7.5% Na_2CO_3 solution. The mixtures were left to stand for 2 h then the absorbance was measured using a spectrophotometer at 750 nm using a gallic acid standard calibration curve. The results were expressed as mg/g gallic acid equivalents (GAE). For determination of HPPs, BGN SDF samples (250 mg) were dissolved in 10 mL distilled water, centrifuged (4000 x *g*, 5 min, 21 °C) and the supernatant was subjected to the Folin–Ciocalteu assay. Tannins were determined by treating IDF samples (250 mg) with a 1:1 mixture of 5 mL/L HCl-Butanol. The mixture was incubated at 100 °C for 1 h then centrifuged (4000 x *g*, 5 min, 21 °C). Tannins were calculated from the anthocyanidin solutions absorbance at a wavelength of 555 nm using a standard curve of 0.0072 ppm and an absorbance of +0.0072.

Assessment of neutral sugars and uronic acids in BGN fibres

BGN fibres were subjected to acid hydrolysis prior to analysis of neutral sugars and uronic acids. SDFs were hydrolysed with 1 M H_2SO_4 at 100 °C for 90 min and IDFs were first hydrolysed with 12 M H_2SO_4 at 30 °C for 90 min and then with 1 M H_2SO_4 at 100 °C for 90 min to yield monomers. After hydrolysis, samples were centrifuged (3000 x *g,* 15 min, 21 °C). IDF residues were washed twice with 2 mL distilled water and SDFs were filtered to remove any suspensions. Uronic acids and neutral sugars were then analysed in the IDF and SDF supernatants by spectrophotometry (340 nm) using K-Arga, K-Fucose, K-Mangl, K-Rhan, K-Uronic and K-Xylose assay kits (Megazyme International, Wicklow, Ireland).

Data analysis

For statistical analysis, IBM Statistical Package for the Social Science (IBM SPSS, version 22) was used. The results were subjected to Multivariate Analysis of Variance (MANOVA) to determine mean differences between treatments. Duncan's multiple range test was conducted to separate mean differences where differences existed.

Results and Discussion

Yield of BGN fibres

Soluble and insoluble dietary fibres were successfully isolated from four varieties of BGN using the modified wet milling method (Figure 1) and the yield of each dietary fibre is given in Table 1. Scanning electron micrographs of BGN fibres are shown in Figure 2. The yield of SDFs was in the range 15.4% (red) to 17.1% (brown-eye) and that of IDFs was in the range 12.0% (brown-eye) to 15.6% (red). There was no significant difference ($p>0.05$) in the yield of SDFs as well as among the IDFs.

Using the enzymatic-gravimetric method to extract DFs from legumes, a lower yield of both IDFs and SDFs has been reported. The lower yield of DFs obtained using the enzymatic-gravimetric-method may be attributed

to the fact that chemicals used in this method result in the loss of some IDFs and most SDFs.[18] The yield of BGN DFs in this study was considered high as several researchers have reported BGN DF content in the range 5.2% to 6.4%.[19-21] The variations in yield among researchers may be attributed to differences in BGN varieties, climatic conditions, type of soil grown on, processing and determination methods used. The SDF content of most edible legumes such as pea, broad pea and soybean cotyledons range between 3.3% and 13.8%.[10,22] The yield of SDFs in this study was higher than the reported range. This increase in yield could be an indication that BGN has a higher SDF content than previously studied legumes. In addition, the use of different extraction methods could be responsible for the differences in yield; the wet milling method could have favoured the extraction of soluble fractions of DF more than other methods.

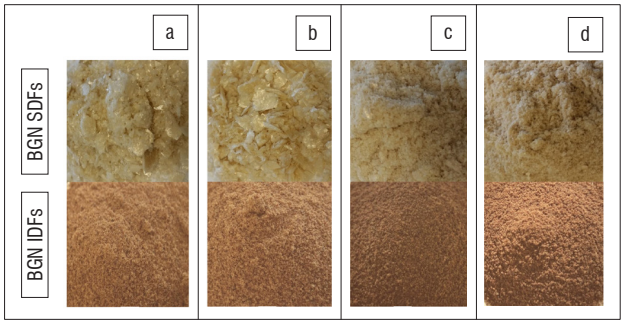

Figure 1: Soluble and insoluble dietary fibres (SDFs and IDFs, respectively) isolated from four varieties of Bambara groundnut (BGN): (a) black-eye, (b) brown-eye, (c) brown and (d) red.

Hydration properties of BGN dietary fibres

Swelling capacity of BGN fibres

The swelling capacity of IDFs ranged from 6.50 mL/g (brown-eye) to 5.50 mL/g (brown) as shown in Table 1. There were no significant ($p>0.05$) differences between brown and red IDFs and also among black-eye, brown-eye and red IDFs in terms of swelling capacity. Brown-eye and black-eye IDFs had higher ($p\leq0.05$) swelling capacities than brown and red IDFs. Swelling capacities in the range 4.28–5.51 mL/g were reported for chickpea and pea IDFs.[5,23] These values are comparable to those obtained in this study. A swelling capacity of 5.51 mL/g was reported for mung bean hulls sieved through a 50 μm mesh which is similar to the sieve size used in this study.[24] The similarities in the results of the two studies suggest that particle size plays a major role in determining the swelling capacity of fibres.[24] Increasing swelling capacity with decreasing particle size has been reported.[24] Thus it can be deduced that particle size has an inverse relationship with swelling capacity.

The swelling capacities of BGN fibres are comparable to that of cellulose (6.2 mL/g), a dietary fibre constituent that is widely used in food products as a bulking agent, stabiliser, thickener and anti-caking agent owing to its hydration properties.[25] Reduced cooking losses, decreased firmness, decreased adhesiveness and reduced stickiness in pea and inulin fibre enriched pastas have been reported.[26] The researchers reported that the increase in swelling capacities of the fibres imparted these desirable characteristics in the pastas.

This study suggested that BGN fibres would make suitable substitutes for cellulose, inulin and pea fibres in food systems such as pasta because of to their swelling capacities. Physiologically, the swelling capacity of fibres is important in the control of blood glucose levels and also contributes to proper gut function.[7,9]

Water holding capacity of BGN fibres

The water holding capacity (WHC) of BGN fibres ranged from 2.41 g water/g sample (red) to 2.84 g water/g sample (black-eye) as shown in Table 1. The WHCs of black-eye and brown-eye IDF were significantly ($p\leq0.05$) higher than the WHC of brown IDF and brown IDF WHC in turn was higher ($p\leq0.05$) than red IDF WHC.

The WHC of passion fruit seed IDF was reported as 2.37 g water/g sample.[27] Passion fruit seed fibre has been described as a functional ingredient that improves the health and functioning of the gut attributing these characteristics to its WHC.[28,29] As the WHC of BGN IDF is comparable to that of passion fruit seed IDF, it can be deduced that BGN IDF can play a similar physiological role.

Several researchers reported the WHC of various legumes in the range 3.13 g water/g sample to 13.4 g water/g sample.[5,13,23,24] The higher values obtained by these researchers could be because of different particle sizes, as well as differences in the chemical nature, composition and processing history of the fibres.[30] The differences can also be attributed to the difference in legume species. The WHC of BGN fibres could find use in the meat, dairy and bakery industries.[31]

Densities of BGN fibres

The bulk and direct densities of BGN SDFs and IDFs were evaluated and the findings are given in Table 1. SDFs had bulk densities in the range 0.46 g/mL (black-eye and brown-eye) to 0.57 g/mL (brown) and direct

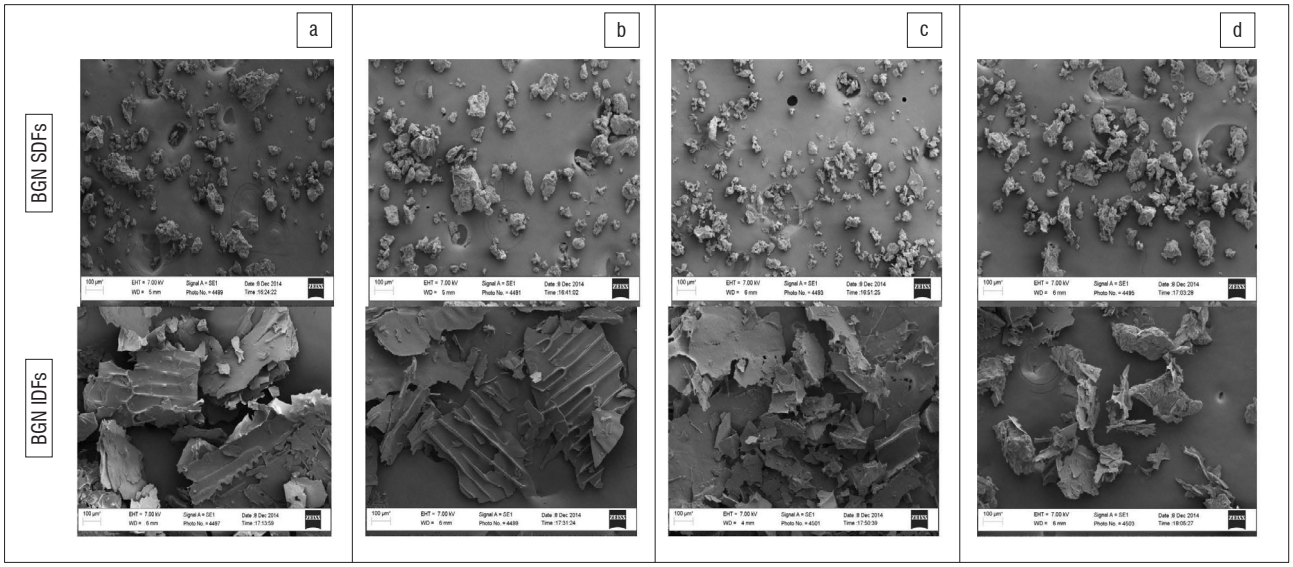

Figure 2: Scanning electron micrographs of soluble and insoluble dietary fibres (SDFs and IDFs, respectively) isolated from four varieties of Bambara groundnut (BGN). (a) black-eye, (b) brown-eye, (c) brown, (d) red.

Table 1: Physicochemical properties of Bambara groundnut dietary fibres

	Yield (%)	Swelling capacity (mL/g)	Water holding capacity (g/g)	Direct density (g/mL)	Bulk density (g/mL)	Oil binding capacity (g/g)	Hydrolysable polyphenols (mg/g)	Tannins (mg/g)
Soluble dietary fibre								
Black-eye	15.4 ± 1.7^{a}	-	-	0.05 ± 0.0^{a}	0.46 ± 0.0^{a}	3.84 ± 0.0^{a}	19.87 ± 0.5^{a}	-
Brown-eye	17.1 ± 3.3^{a}	-	-	0.06 ± 0.0^{a}	0.46 ± 0.0^{a}	4.03 ± 0.1^{b}	20.86 ± 0.0^{b}	-
Brown	15.4 ± 0.9^{a}	-	-	0.11 ± 0.0^{a}	0.57 ± 0.0^{a}	3.72 ± 0.1^{a}	6.89 ± 0.2^{c}	-
Red	17.1 ± 0.6^{a}	-	-	0.07 ± 0.0^{a}	0.50 ± 0.0^{a}	2.78 ± 0.1^{c}	8.26 ± 0.3^{d}	-
Insoluble dietary fibre								
Black-eye	13.1 ± 2.5^{a}	6.17 ± 0.3^{a}	2.84 ± 0.1^{a}	0.53 ± 0.0^{a}	0.57 ± 0.0^{ab}	1.40 ± 0.7^{a}	10.96 ± 0.2^{a}	2.10 ± 0.0^{a}
Brown-eye	12.0 ± 1.4^{a}	6.50 ± 0.0^{a}	2.83 ± 0.1^{a}	0.50 ± 0.0^{ab}	0.67 ± 0.0^{c}	1.40 ± 0.2^{a}	11.44 ± 0.3^{a}	2.07 ± 0.1^{a}
Brown	15.6 ± 0.9^{a}	5.50 ± 0.0^{b}	2.60 ± 0.0^{b}	0.45 ± 0.0^{c}	0.57 ± 0.0^{a}	1.46 ± 0.6^{a}	14.43 ± 0.6^{a}	1.19 ± 0.6^{b}
Red	13.7 ± 2.7^{a}	6.00 ± 0.5^{ab}	2.41 ± 0.1^{c}	0.49 ± 0.0^{b}	0.58 ± 0.0^{b}	1.52 ± 0.0^{a}	13.27 ± 1.6^{a}	1.36 ± 1.6^{c}

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly ($p>0.05$) different.

densities in the range 0.05 g/mL (black-eye) to 0.11 g/mL (brown). Red SDF was significantly ($p\leq0.05$) higher than both brown-eye and black-eye SDFs but lower ($p\leq0.05$) than brown SDF in terms of bulk density while all SDFs differed significantly ($p\leq0.05$) in direct density. IDFs had bulk densities in the range 0.57 g/mL (red and black-eye) to 0.67 g/mL (brown-eye) and direct densities in the range 0.45 g/mL (brown) to 0.53 g/mL (black-eye). The IDFs of black-eye and brown-eye as well as black-eye and red varieties did not differ significantly ($p>0.05$) in their direct densities. Brown IDF had a significantly ($p\leq0.05$) lower direct density than the other three fibres.

Direct density is measured without compressing the fibres while bulk density is measured after compressing the fibres. Consequently, bulk density measurements yielded higher values than direct densities. Dalgetty and Baik[5] concluded that SDFs have higher densities than IDFs. The results obtained in the current study disagree with Dalgetty and Baik's[5] conclusion as IDFs had higher bulk and direct densities than SDFs. The bulk densities of BGN IDFs were comparable to those of IDFs from passion fruit seeds (0.68 g/mL), soybean (0.43 g/mL), peas (0.54–0.56 g/mL), pigeon pea (0.47 g/mL) and chickpea (0.65 g/mL).[27,32] These fibres are commercially available hence this is one of the indications that BGN dietary fibres have the potential to successfully compete commercially with other fibres. Differences in bulk densities with other leguminous fibres could be because of different structural compositions owing to different leguminous species.[13]

Density is of importance in packaging, with higher densities resulting in a reduced ability to compress. Therefore, the densities of BGN fibres could be an advantage as they will pack closely together, hence requiring less packaging material, resulting in cost saving.[15]

Oil binding capacity of BGN fibres

The oil binding capacities (OBCs) of SDFs ranged from 2.78 g oil/g sample (brown) to 4.03 g oil/g sample (brown-eye) (Table 1). Black-eye and red SDFs did not differ significantly in OBC, brown-eye SDF was significantly higher than the other three SDFs and brown SDF was significantly lower than all three SDFs. Among the IDFs, brown IDF had the highest OBC of 1.52 g oil/g sample, while brown-eye and black-eye IDFs both had the lowest OBC of 1.40 g oil/g sample. All four IDFs did not differ ($p>0.05$) in terms of OBC. SDFs showed higher OBCs than IDFs.

Higher IDF OBC values were reported for pea (6.93 g oil/g sample), chickpea (4.25 g oil/g sample) and lentil fibres (4.01 g oil/g sample)[5]. These differences can be attributed to structural differences and compositional variation of the fibres. OBC of 1.49 g oil/g sample to 1.83 g oil/g sample were reported for mung bean hulls which compares fairly with the OBC of BGN IDFs.[24]

A total of 11 commercial fibres were studied and low OBC values were reported with the highest being 0.02 g oil/g sample from bamboo.[25] The low OBC values indicated that BGN fibres can compete commercially with other fibres in stabilising high fat foods and emulsions.[23] Lower OBC values for SDFs derived from pea (1.15 g oil/g sample), chickpea (1.14 g oil/g sample) and lentil fibres (0.89 g oil/g sample) have been reported.[5]

This study indicated that the use of BGN fibres would be economical as less BGN fibre would be used to render the desirable properties compared to other leguminous fibres. The ability of fibres to bind oil can be harnessed by the food industry to reduce fat losses upon cooking and in stabilising emulsions.[23,33] Physiologically, the OBCs of BGN fibres would allow them to play a role in bile acid absorption and consequently cholesterol reduction.[30] OBC would also be significant in reducing fat absorption by the body.

Colour characteristics of BGN fibres

The colour attributes of BGN measured were lightness (L*), redness (+a*) / greenness (-a*), blueness (-b*) / yellowness (+b*), hue and chroma (Table 2). SDFs were lighter than IDFs and all the BGN fibres had +a* and +b* values indicating that they were more associated with redness and yellowness. The redness and yellowness of these fibres suggests their antioxidant properties.[34,35] BGN fibres are high in polyphenolic compounds (Table 2). Chroma describes the vividness or dullness of a colour and hue is how the colour of an object is perceived.[34] The hue angle of BGN DFs indicated a yellowish-red colour associated with these fibres.

Lightness of dietary fibres is of importance in food products as it determines the extent to which the original colour of the food is affected.[25,30] The varying colours of the BGN dietary fibres are advantageous as the manufacturer will have a choice of a fibre variety that best suits the colour of their product. IDFs had darker colours hence could find use in products such as meat emulsions and brown bread, while the lighter coloured SDFs could be used in food products such as white bread and beverage emulsions.

A colour difference (ΔE) of 1 is the threshold at which a trained observer would notice the difference between two colours, a ΔE between 4 and 8 is deemed acceptable and above 8 is deemed unacceptable and likely to be rejected by consumers.[36] Table 3 gives the colour difference between BGN fibres. All the SDFs showed acceptable differences with ΔE ranging between 0.81 and 3.08.

Table 2: Colour attributes of Bambara groundnut dietary fibres

	Lightness	Redness/greenness	Yellowness/blueness	Chroma	Hue (°)
Insoluble dietary fibre					
Black-eye	36.6 ±0.4[a]	9.9 ±0.1[a]	17.6 ±0.3[a]	20.2 ±0.3[a]	60.6 ±0.3[a]
Brown-eye	37.9 ±0.5[b]	10.1 ±0.1[a]	18.5 ±0.2[b]	21.1 ±0.2[a]	61.5 ±0.2[b]
Brown	24.3 ±0.1[c]	6.0 ±0.1[b]	7.9 ±0.1[c]	10.0 ±0.1[b]	52.8 ±0.4[c]
Red	30.8 ±0.2[d]	7.9 ±0.1[c]	10.5 ±0.2[d]	12.7 ±0.9[c]	52.8 ±0.1[c]
Soluble dietary fibre					
Black-eye	73.0 ±0.2[ab]	1.7 ±0.0[a]	13.8 ±0.1[a]	13.9 ±0.1[a]	83.1 ±0.1[a]
Brown-eye	74.0 ±0.5[a]	2.4 ±0.0[b]	15.5 ±0.4[b]	15.7 ±0.1[b]	81.2 ±0.5[ab]
Brown	71.7 ±1.1[bc]	2.3 ±0.3[b]	15.5 ±0.3[b]	15.9 ±0.8[b]	81.1 ±1.8[ab]
Red	71.0 ±1.3[c]	2.5 ±0.2[b]	15.6 ±0.5[b]	16.3 ±1.0[b]	79.8 ±1.4[b]

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly ($p>0.05$) different.

Hence, they could be used interchangeably in products without a noticeable difference. IDFs, with the exception of black-eye – brown-eye and brown – red comparisons had ΔE above 8 meaning their colour differences were very apparent and if used interchangeably, a perceivable difference would be expected.

Table 3: Colour differences (ΔE) between Bambara groundnut dietary fibres

Variety	Insoluble dietary fibre ΔE	Soluble dietary fibre ΔE
Black-eye – brown-eye	1.55	2.12
Black-eye – brown	16.14	2.22
Black-eye – red	18.66	2.81
Brown-eye – brown	17.67	2.31
Brown-eye – red	10.94	3.08
Brown – red	7.20	0.81

Phenolic content of BGN fibres

The antioxidant capacity of BGN fibres, as represented by hydrolysable phenols (HPPs) and tannins, is shown in Table 1. The HPP content of SDFs ranged from 6.89 mg/g GAE (brown) to 20.86 mg/g GAE (brown-eye). All four SDFs differed significantly in their HPP content. The HPP content of IDFs ranged from 10.96 mg/g GAE (black-eye) to 14.43 mg/g GAE (brown). Black-eye and brown-eye IDFs as well as brown and red IDFs did not differ significantly in their HPP content. The tannin content of IDFs ranged from 1.12 mg/g (black-eye) to 2.1 mg/g (brown). Black-eye and brown-eye IDFs were both significantly higher ($p\leq0.05$) in tannin content than red and brown IDFs. Brown IDF was significantly lower than the other three fibres in terms of tannin content. The difference in phenolic content of the BGN fibres was in agreement with Nti[35] who reported that tannin content differs from one BGN variety to another.

The high polyphenolic composition of BGN reveals their potential antioxidant properties. It has been suggested that fibres can be exploited as novel antioxidants and would be of importance in protecting against superoxide radicals, hydroxyl free radicals and lipid peroxidation.[23] They would thus find use as ingredients in fatty foodstuffs to improve oxidative stability, hence improving their shelf life. Antioxidants are important for human health as they prevent some degenerative diseases like cancers and decrease the oxidation of low density lipoproteins, thereby avoiding arteriosclerosis and related coronary heart diseases.[37-39] Antioxidants carry out these functions by reacting with free radicals forming stable or non-reactive radicals.[39] BGN fibres can be a useful source of natural antioxidants as alternatives to artificial antioxidants; artificial antioxidants have been shown to be carcinogenic and teratogenic.[39] The low tannin content observed in BGN fibres could be advantageous as tannins have been associated with anti-nutritional properties because of their ability to form complexes with some nutrients, including divalent minerals and proteins, rendering them bio-unavailable.[15,40]

Neutral sugars and uronic acids in BGN fibres

Seven neutral sugars were analysed for in BGN fibres and the results are given in Table 4. Arabinose and galactose coeluted in this study and therefore are presented as arabinose/galactose in Table 4. The percentage of arabinose/galactose in SDFs was in the range 9.4% (brown) to 19.6% (black-eye). The percentage of fructose in SDFs ranged from 1.3% (black-eye) to 1.7% (brown). Fucose and glucose were obtained in low amounts (below 1%) in SDFs while relatively higher percentages of xylose were obtained in the range 13.0% (brown) to 16.6% (black-eye) (Table 4). Brown-eye and black-eye SDFs did not differ significantly ($p>0.05$) in their sugar composition with the exception of arabinose/galactose. Rhamnose was absent in all SDFs. This finding is in agreement with Dalgetty and Baik[5] who reported the absence of rhamnose in SDFs of pea, lentil and chickpea. The researchers also reported the absence of arabinose and mannose in SDFs. In the current study however, these two sugars were present. These authors further reported higher values of xylose (32%) in chickpea SDF. These differences can be attributed to the different analytical methodologies adopted, with sugar assay having been used in the current study and HPLC having been used in the study by Dalgetty and Baik.[5]

The presence of these sugars in BGN SDFs suggests the possible presence of galactomannans, arabinoxylans and arabinogalactans. Galactomannans are related to locust bean and guar gums and their solubility in water increases with increasing galactose content. BGN SDFs had higher quantities of galactose compared to mannose hence the solubility of galactomannans would be elevated.[41] Arabinoxylans possess antioxidant capabilities and influence water balance and rheology[42] and arabinogalactans possess similar characteristics as gum Arabic.[43-45] The suggestive presence of these hydrocolloids in BGN fibres could mean that BGN fibres possess similar beneficial characteristics and thus can be classified with them.

Table 4: Neutral sugar and uronic acid composition of Bambara groundnut dietary fibres

	SUGARS (%)							
Variety	**Arabinose/ Galactose**	**Fructose**	**Fucose**	**Glucose**	**Mannose**	**Rhamnose**	**Xylose**	**Uronic acids**
Soluble dietary fibre								
Black-eye	19.6 ± 0.9^{a}	1.3 ± 0.1^{a}	0.3 ± 0.1^{a}	1.0 ± 0.1^{a}	6.1 ± 0.5^{a}	0	16.6 ± 1.6^{a}	11.5 ± 1.0^{a}
Brown-eye	15.2 ± 1.5^{b}	1.5 ± 0.1^{ab}	0.3 ± 0.1^{a}	0.8 ± 0.1^{ab}	6.5 ± 0.2^{a}	0	15.6 ± 0.2^{ab}	10.2 ± 0.8^{a}
Brown	9.4 ± 0.1^{c}	1.7 ± 0.1^{b}	0.1 ± 0.0^{b}	0.8 ± 0.1^{ab}	7.4 ± 0.6^{b}	0	13.0 ± 1.8^{c}	10.6 ± 0.3^{a}
Red	10.0 ± 0.6^{c}	1.6 ± 0.1^{b}	0.3 ± 0.0^{a}	0.6 ± 0.2^{b}	5.0 ± 0.3^{c}	0	13.7 ± 0.8^{bc}	11.5 ± 1.1^{a}
Insoluble dietary fibre								
Black-eye	2.8 ± 0.2^{a}	1.7 ± 0.1^{ac}	0.1 ± 0.0^{a}	0.6 ± 0.1^{a}	4.7 ± 0.4^{a}	2.6 ± 0.1^{a}	11.3 ± 1.2^{a}	6.7 ± 0.7^{a}
Brown-eye	2.3 ± 0.1^{b}	1.7 ± 0.1^{a}	0.3 ± 0.1^{b}	0.7 ± 0.1^{ab}	6.6 ± 0.1^{ab}	1.0 ± 0.1^{b}	11.3 ± 1.9^{a}	8.6 ± 0.7^{b}
Brown	2.5 ± 0.2^{ab}	1.5 ± 0.1^{b}	0.4 ± 0.1^{b}	0.9 ± 0.1^{c}	5.6 ± 0.4^{c}	1.2 ± 0.1^{c}	13.8 ± 2.3^{a}	10.5 ± 0.6^{ab}
Red	2.8 ± 0.2^{a}	1.9 ± 0.1^{c}	0.2 ± 0.1^{a}	0.8 ± 0.0^{b}	5.6 ± 0.6^{b}	1.1 ± 0.1^{c}	12.4 ± 0.8^{a}	10.6 ± 1.0^{a}

Values are mean ± standard deviation. Means within a column followed by the same superscript are not significantly ($p>0.05$) different.

The uronic acid content of SDFs did not differ significantly ($p>0.05$) and ranged from 10.6% (brown) to 11.5% (red). Dalgetty and Baik[5] reported lower uronic acids in pea, lentil and chickpea SDFs in the range 0.2% to 1.3%. Uronic acids form salts with some wastes in the human body thereby facilitating their excretion.[46] Hence, their presence in BGN DFs would contribute to the body's detoxification.

In IDFs, arabinose/galactose was in the range 2.3% (brown-eye) to 2.8% (red). The percentage of fructose in IDFs ranged from 1.5% (brown) to 1.9% (red). Fucose and glucose were obtained in low amounts (below 1%) while rhamnose ranged from 1.0% (brown-eye) to 2.6% (black-eye). Relatively high percentages of xylose were obtained in IDFs (Table 4). The presence of these in BGN IDFs is an indication of the presence of some polysaccharides such as cellulose (glucose) and hemicellulose (xylose, glucose, arabinose, galactose and mannose).[15,47]

The presence of these sugars in BGN IDFs suggests the presence of some polysaccharides such as pectic substances (rhamnose and galactose)[24] which in turn suggests the presence of rhamnogalacturonans.[15] Rhamnogalacturonans have been reported to bind heavy metals in the human body as well as lower blood cholesterol.[43,44] The presence of arabinose and xylose in IDFs suggest the presence of arabinoxylans. Low quantities of arabinose and galactose in IDFs suggest low quantities of arabinogalactans.

The uronic acid content of IDFs ranged from 6.7% (black-eye) to 10.6% (red). There was no significant difference among the red, brown and black-eye IDFs as well as between brown and brown-eye IDFs in uronic acid content. Dalgetty and Baik[5] reported lower uronic acid of pea, lentil and chickpea IDFs in the range 2.0% to 2.8% indicating the superiority in uronic acid content of BGN IDFs over other leguminous IDFs.

Conclusions

The wet milling method was successfully applied in the extraction of BGN SDFs and IDFs yielding an appreciable amount of both fractions. Black-eye and brown-eye fibres have superior physicochemical properties compared to the brown and red fibres as evidenced by their higher swelling capacities, water-holding capacities, oil binding capacities, phenolic as well as total sugar content. Brown-eye and black-eye IDFs were lighter in colour, yellower, redder, more saturated and had higher hues compared to the red and brown IDFs. The physicochemical properties of BGN fibres make them valuable to the food industry as thickening agents, stabilisers, health ingredients as well as cryoprotectants in frozen dairy products. BGN fibres can be considered suitable alternatives for commercial fibres such as pea, chickpea and lentil fibres as they have been shown to possess similar qualities to these fibres.

Acknowledgements

The authors acknowledge the Cape Peninsula University of Technology (CPUT) University Research Funding and the National Research Foundation for financial assistance towards the research running costs. Bambara groundnut dietary fibre is a patent of CPUT; Dietary fibre supplement: South Africa complete patent (2014/04371).[48]

Authors' contributions

The authors contributed equally to the work presented in this paper. Y.M. was responsible for the experimental work and wrote the manuscript. V.A.J. supervised the project, carried out the statistical analysis and proofread the manuscript.

References

1. Jideani VA, Diedericks CF. Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*. In: Oguntibeju O. editor. Antioxidant-antidiabetic agents and human health. Croatia: InTech; 2014. p. 187. http://dx.doi.org/10.5772/57029

2. Jideani VA, Mpotokwana SM. Modelling of water absorption of Botswana Bambara varieties using Peleg's equation. J Food Eng. 2009;92(2):182–188. http://dx.doi.org/10.1016/j.jfoodeng.2008.10.040

3. Fasoyiro SB, Ajibade SR, Omole AJ, Adeniyan ON, Farinde EO. Proximate, mineral and anti-nutritional factors of some underutilized grain legumes in South-Western Nigeria. Nutr Food Sci. 2006;38:18–23. http://dx.doi.org/10.1108/00346650610642151

4. Fasoyiro S, Yudi W, Taiwo K. Processing and utilization of legumes in the tropics. In: Eissa AA, editor. Trends in vital food and control engineering. Croatia: InTech; 2012.

5. Dalgetty DD, Baik B. Isolation and characterisation of cotyledon fibers from peas, lentils, and chickpeas. Cereal Chem. 2003;80(3):310–315.

6. Ojimelukwe PC. Sourcing and processing of legumes. In: Onwualu PA, Obasi SC, Ukpabi, UJ, editors. Nigerian agro raw materials development. Abuja: Raw Materials Research and Development Council, 2009.

7. Daou C, Zhang H. Physico-chemical properties and antioxidant activities of Dietary Fiber derived from defatted rice bran. Adv J Food Sci Technol. 2011;3(5):339–347.

8. Hawkes C. Uneven dietary development: Linking the policies of globalization with the nutrition transition, obesity, and diet-related chronic diseases. Global Health. 2006;2:4. http://dx.doi.org/10.1186/1744-8603-2-4

9. Wood JA, Grusak MA. Nutritional value of chickpea. In: Yadav SS, Redden R, Chen W, Sharma B, editors. Chickpea breeding and management. Trowbridge: Cromwell Press; 2007. p.119. http://dx.doi.org/10.1079/9781845932138.005

10. Khan AR, Alam S, Ali S, Bibi S, Khalil IA. Dietary fiber profile of food legumes. Sarhad J Agric. 2007;23(3):763–766.

11. USA Dry Pea and Lentil Council. Processing methods for dry peas, lentils and chickpeas. In: Pulse processing technical manual. Moscow: USA Dry Pea and Lentil Council; 2010.

12. Bogracheva T, Cserhalmi Z, Czukor B, Fornal J, Schuster-Gajzago I, Kovacs ET, et al. Processing. In: Henley CL, editor. Carbohydrates in grain legume seeds: Improving nutritional quality and agronomic characteristics. New York: CABI Publishing; 2001. p. 89–92.

13. Tiwari U, Cummins E. Functional and physicochemical properties of legume fibers. In: Tiwari BK, Gowen, A, McKenn B, editors. Pulse foods: Processing, quality and nutraceutical applications. London: Academic Press; 2011. p. 139, 144. http://dx.doi.org/10.1016/B978-0-12-382018-1.00005-8

14. Urriola PE, Cervantes-Pahm SK, Stein HH. Fiber in swine nutrition. In: Chiba LI, editors. Sustainable swine nutrition. West Sussex: Wiley-Blackwell; 2013. p. 259. http://dx.doi.org/10.1002/9781118491454.ch11

15. Diedericks CF. Functional properties of Bambara groundnut (*Vigna Subterranea* (L.) Verdc.) non-starch polysaccharides in model and food systems [MTech dissertation]. Cape Town: Cape Peninsula University of Technology; 2014.

16. Wang N, Toews, R. Certain physicochemical and functional properties of fibre fractions from pulses. Food Res Int. 2011;44(8):2515–2523. http://dx.doi.org/10.1016/j.foodres.2011.03.012

17. Parrott ME, Thrall BE. Functional properties of various fibers: Physical properties. J Food Sci. 1978;43:759–763. http://dx.doi.org/10.1111/j.1365-2621.1978.tb02412.x

18. Gordon DT, Okuma K. Determination of total dietary fiber in selected foods containing resistant maltodextrin by enzymatic-gravimetric method and liquid chromatography: Collaborative study. J AOAC Int. 2002;85:435–444.

19. Baryeh EA. Physical properties of Bambara groundnuts. J Food Eng. 2001;47:321–326. http://dx.doi.org/10.1016/S0260-8774(00)00136-9

20. Mkandawire CH. Review of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) production in Sub-Sahara Africa. Agric J. 2007;2(4):464–470.

21. Murevanhema YY, Jideani VA. Potential of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk as a probiotic beverage: A review. Crit Rev Food Sci. 2013;53(9):954–967. http://dx.doi.org/10.1080/10408398.2011.574803

22. Guillon F, Champ M. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. Food Res Int. 2002;33:233–245. http://dx.doi.org/10.1016/S0963-9969(00)00038-7

23. Elleuch M, Bedigian D, Roiseux O, Besbes S, Blecker C. Dietary fibre and fibre-rich by-products of food processing; Characterisation technological functionality and commercial applications: A review. Food Chem. 2011;124:411–421. http://dx.doi.org/10.1016/j.foodchem.2010.06.077

24. Huang SC, Lia TS, Cheng TC, Chan HY, Hwang SM, Hwang DF. *In vitro* interactions on glucose by different fibre materials prepared from mung bean hulls, rice bran and lemon pomace. J Food Drug Anal. 2009;17:307–314.

25. Rosell CM, Santos E, Collar C. Physico-chemical properties of commercial fibres from different sources: A comparative approach. Food Res Int. 2009;42:176–184. http://dx.doi.org/10.1016/j.foodres.2008.10.003

26. Tudorica CM, Kuri V, Brennan CS. Nutritional and physicochemical characteristics of dietary fiber enriched pasta. J Agric Food Chem. 2002:50(2):347–356. http://dx.doi.org/10.1021/jf0106953

27. Chau CF, Huang YL. Characterization of passion fruit seed fibres - a potential fibre source. Food Chem. 2004;85:189–194. http://dx.doi.org/10.1016/j.foodchem.2003.05.009

28. Chau CF, Huang YL, Chang FY. Effects of fibre derived from passion fruit seed on the activities of ileum mucosal enzymes and colonic bacterial enzymes in hamsters. J Sci Food Agric. 2005;85:2119–2124. http://dx.doi.org/10.1002/jsfa.2230

29. Esposito F, Arlotti G, Bonifati AM, Napolitano A, Vitale D, Fogliano V. Antioxidant activity and dietary fibre in durum wheat bran by-products. Food Res Int. 2005;38:1167–1173). http://dx.doi.org/10.1016/j.foodres.2005.05.002

30. Tosh SM, Yada S. Dietary fibres in pulse seeds and fractions: Characterisation, functional attributes and applications. Food Res Int. 2010;43(2):450–460. http://dx.doi.org/10.1016/j.foodres.2009.09.005

31. Kohajdova Z, Karovicova J, Magala M. Effect of lentil and bean flours on rheological and baking properties of wheat dough. Chem Pap. 2013;67(4):398–407. http://dx.doi.org/10.2478/s11696-012-0295-3

32. Maskus H. Pulse processing, functionality and application. Manitoba: University of Winnipeg; 2010.

33. Slavin J. Fiber and prebiotics: Mechanisms and health benefits. Nutrients. 2013;5(4):1417–1435. http://dx.doi.org/10.3390/nu5041417

34. Murevanhema YY. Evaluation of Bambara groundnut (*Vigna subterranea* (L.) Verdc) milk fermented with lactic acid bacteria as a probiotic beverage [MTech dissertation]. Cape Town: Cape Peninsula University of Technology; 2012.

35. Nti CA. Effects of Bambara groundnut (*Vigna subterranea*) variety and processing on the quality and consumer appeal for its products. Int J Food Sci Tech. 2009;44(11):2234–2242. http://dx.doi.org/10.1111/j.1365-2621.2009.02064.x

36. Sharma A. Understanding color management. USA: Thomson Delmar Learning, 2004.

37. Tomas M, Latorre G, Senti M, Marrugat J. The antioxidant function of high density lipoproteins: A new paradigm in atherosclerosis. Rev Esp Cardiol. 2004;57(6):557–569. http://dx.doi.org/10.1016/s1885-5857(06)60630-0

38. Tsimikas S. Lipoproteins and oxidation. In: Bourassa MG, Tardif JC, editors. Antioxidants and cardiovascular disease. 2nd ed. Montreal: Springer; 2006. p 17. http://dx.doi.org/10.1007/0-387-29553-4_2

39. Betancur-Ancona D, Perza-Mercado G, Moguel-Ordonez Y, Fuertes-Blanco S. Physicochemical characterisation of lima beans (*Phaselous lunatus*) and jack bean (*Canavalia ensiformis*) fibrous residues. Food Chem. 2004;84:287–295. http://dx.doi.org/10.1016/S0308-8146(03)00213-9

40. Saura-Calixto FD, Bravo L. Dietary fiber-associated compounds: Chemistry, analysis, and nutritional effects of polyphenols. In: Cho SS, Dreher ML. editors. Handbook of dietary fiber. New York: Marcel Dekker Inc; 2001. http://dx.doi.org/10.1201/9780203904220.ch22

41. Silveira JLM, Bresolin TMB. Pharmaceutical use of galactomannans. Quim Nova. 2011;34(2):292–299. http://dx.doi.org/10.1590/S0100-40422011000200023

42. Saeed F, Pasha I, Anjum FM, Sultan MT. Arabinoxylans and arabinogalactans: A comprehensive treatise. Crit Rev Food Sci Nutr. 2011;51(5):467–476. http://dx.doi.org/10.1080/10408391003681418

43. Sivam AS, Sun-Waterhouse D, Quek SY, Perera CO. Properties of bread dough with added fiber polysaccharides and phenolic antioxidants: A review. J Food Sci. 2010;75(8):163–174. http://dx.doi.org/10.1111/j.1750-3841.2010.01815.x

44. Khotimchenko Y, Khozhaenko E, Kovalev V, Khotimchenko M. Cerium binding activities of pectins isolated from the seagrasses *Zostera marina* and *Phyllospadix iwatensis*. Mar Drugs. 2012;10(4):834–848. http://dx.doi.org/10.3390/md10040834

45. Golenser J, Frankenburg S, Ehrenfreund T, Domb AJ. Efficacious treatment of experimental leishmaniasis with amphotericin B-arabinogalactan water-soluble derivatives. Antimicrob Agents Chemother. 1999;43(9):2209–2214.

46. Vazquez JA, Rodriguez-Amado I, Montemayor MI, Fraguas J, Gonzalez MDP, Murado MA. Chondroitin sulfate, hyaluronic acid and chitin/chitosan production using marine waste sources: Characteristics, applications and eco-friendly processes: A review. Mar Drugs. 2013;11(3):747–774. http://dx.doi.org/10.3390/md11030747

47. Dhingra D, Michael M, Rajput H, Patil RT. Dietary fibre in foods: A review. J Food Sci Technol. 2012;49:255–266. http://dx.doi.org/10.1007/s13197-011-0365-5

48. Jideani VA, Diedericks CF. Dietary fibre supplement from Bambara groundnuts (*Vigna subteranea*). South Africa – Complete patent (2014/04371); 2014 June 19. Pretoria: Companies and Intellectual Property Division; 2014

Appendix C: Book of abstracts – Research outputs presented at national and international conferences

1. **Maphosa, Y**. & Jideani, V. A. (2014). Dietary fibre extraction from plant materials - A review U6 Consortium 2nd International Conference, Cape Town, South Africa, 6 - 10 September 2014. Pp. 26. (Paper presentation).

2. **Maphosa, Y**. & Jideani, V. A. (2014). Polyphenolic and neutral sugar composition of Bambara Groundnut non-starch polysaccharides. CPUT Postgraduate Conference, Bellville, Cape Town, South Africa, 5 November 2014. Pp 1. (Paper presentation).

3. **Maphosa, Y**. & Jideani, V. A. (2015). Stability and rheological properties of orange-oil beverage emulsion stabilised with Bambara groundnut soluble dietary fiber. Institute of Food Technologists, IFT15 International Conference, Chicago, Illinois, USA, 11 - 14 July, 2015. (Poster presentation).

4. **Maphosa, Y**. & Jideani, V. A. (2015). Rheological and thermal characteristics of Bambara groundnut non-starch polysaccharides. 21st South African Association of Food Science and Technology (SAAFoST) Biennial International Congress and Exhibition 2015, Durban, South Africa, 6 - 9 September 2015. (Paper presentation).

5. **Maphosa, Y**. & Jideani, V. A. (2016). Effect of Bambara groundnut soluble dietary fibres on the rheological properties of orange oil beverage emulsions. 4th International ISEKI_Food Conference 2016, Vienna, Austria, 6 - 8 July, 2016 (Poster presentation).