METABOLITE PROFILE OF BAMBARA GROUNDNUT MALTING PROCESS AND CHARACTERISTICS OF ITS MALT EXTRACTS

by

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DECLARATION

I, **Adeola Helen Adetokunboh**, hereby declare that the work contained in the research report is my own original work and has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions, not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

Bambara groundnut *(Vigna subterranea)* [BGN] is a hardy, drought-resistant crop, termed a complete food because of its high nutritional value, with an average protein, carbohydrate, and fat contents of 17.5%, 64.0% (dry matter), and 6.5%, respectively. BGN has been sprouted to know its nutritional content and functional characteristics in food application and product development. Sprouting facilitates the breakdown of macronutrients and antinutritional compounds in seeds and synthesis metabolites. Recently, metabolomics has been considered an effective tool for profiling metabolites. However, despite the studies on BGN seeds, there is little or no studies on the enzyme, total polyphenolics, antioxidant activities and metabolites of malted BGN as affected by steeping and sprouting times. Bambara groundnut was malted by steeping in distilled water at 25 ± 3^{o}C for 36 and 48 h. The steeped seeds were sprouted for 144 h at 30^{o}C, and samples were drawn every 24 h for drying. The dried samples were analysed for physicochemical properties (sprout length, moisture, pH, colour, protein content), enzymes, antioxidant activities and metabolites. The base malt (BGN-BM) was produced by drying sprouted BGN (green malt) at 50^{o}C for 24 h. The toasted malt (BGN-TM) was produced from the base malt (dried at 50^{o}C) subjected to 170^{o}C for 30 min. The caramel malt (BGN-CM) was produced from the green malt heated to 80^{o}C for 1 h and gradually increased every 20 min to 120^{o}C for 2 h. Roasted malt (BGN-RM) was produced by drying green malt at 180^{o}C for 1 h. Syrups were produced isothermally from the speciality malts. The speciality malts and syrups were analysed for colour, pH, protein content, α and β-amylases, total polyphenols, antioxidants, and metabolites. Sprout length was measured using the vernier caliper, pH by the laboratory pH meter. The colour was measured by the colour Flex EZ 25 mm aperture set for daylight illumination D65 and 10° standard observer angle. The Dumas Nitrogen analysis was used to determine the protein content, and crude protein was calculated by multiplying the measured nitrogen by the protein factor of 6.25 expressed in percentage. The α- and β-amylase activities of the sprouted samples were analysed by the enzymatic Ceralpha Method (K-CERA, Megazyme) kit and enzymatic Beta-Amylase (Megazyme, K-BETA3) kit, respectively. The Folin–Ciocâlteu reagent (FCR), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay methods were used to determine the polyphenolic and antioxidants activities. The metabolites were analysed using Gas Chromatography-Mass Spectrometry (GC-MS) for acids, sugars, sugar alcohols and amino acids. Gas Chromatography-Flame Ionization Detection (GC-FID) for Fatty acid methyl esters (FAME) and Headspace Gas Chromatography-Flame Ionization Detector (HS-GC-FID) for the volatiles. The moisture of the steeped seeds increased with steeping times. There was a significant difference ($p = 0.004$) between the 36 and 48 h steeped BGN seeds sprouts length, with 36 h steeped

sprouts longer. The steeping and sprouting times significantly affected the BGN malt colour quality and pH. There was no significant difference in the protein content of the BGN seeds based on steeping and sprouting times. The activity of amylases differed significantly for the 36 and 48 h steeping time, with mean α-amylase of 0.16 and 0.15 CU/g, respectively, β-amylase of 0.22 and 0.23 BU/g for the two steeping times. The optimal condition for malting BGN seeds for an amylase-rich malt was 36 h steeping and 96 h sprouting. The total polyphenolic content increased significantly with increased steeping and sprouting times. There was a significant ($p = 0.000$) difference between FRAP and DPPH antioxidant concentration in the 36 and 48 steeping times; however, sprouting from 24 to 144 h did not show a significant difference. The metabolic components during the malting process of BGN seeds were affected by steeping and sprouting times. The metabolites showed different levels of fatty acid methyl esters (FAME), sugars and organic acids, amino acids, and volatiles in the raw and sprouted BGN seeds, as well as the speciality malts. Fatty acid methyl esters (FAME) were not significantly different for the two steeping times. Linoleic acids were high after 48 and 96 h sprouting for the two steeping regimes. Sugars and acids decreased as steeping time increased while myo-inositol increased. Sugars, acids, and sugar alcohol were significantly different ($p \leq 0.05$) based on sprouting times for the two steeping regimes. The two steeping regimes differed significantly in the amino acid concentration while the essential amino acids increased with sprouting time. An increase in steeping time reduced the volatile concentration, and there were changes in its concentration throughout the sprouting process. Ten volatiles were identified in the raw BGN seeds, 9 volatiles in steeped BGN seeds (36 and 48 h), while 21 and 14 volatiles were detected in the sprouted 36 and 48 h steeped BGN. The volatiles detected consisted of hydrocarbons, organic, alkanes, ketones, and aromatics compounds. The colours of the speciality malts and syrups were significantly ($p = 0.000$) different. The BGN speciality malt showed differences in colour with a decrease in lightness (L*) and increased redness (a*) and yellowness (b*). The BGN speciality malts protein ranged from 6.30 to 6.52. The protein content of the BGN speciality malts was significantly different, while the protein content of the syrups was not significantly different. The amylase activities, total polyphenols and antioxidants of the BGN speciality malts differed for all the BGN speciality malt. Twenty-nine volatiles were detected in the speciality malts with the pyrazine, 2,5-dimethyl, more abundant. Fifteen amino acids consisted of seven essential amino acids, and eight non-essential amino acids were detected. Fatty acid methyl esters (FAME) identified were palmitoleic, oleic, linolelaidic, linoleic and arachidic acid. The sugars, organic acids and sugar alcohols consisted of lactic acid, fructose, sucrose, and myo-inositol. The steeped and sprouted BGN seeds, BGN speciality malts and their extracts exhibited physicochemical characteristics and metabolites that could be used in the household, industries and benefit health-conscious consumers.

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DEDICATION

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GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
Akara	Fritter made from cowpeas or black eye peas found throughout West African
Alpha-amylase	An enzyme that breaks large, complex, insoluble starch molecules into smaller, soluble molecules
AOAC	Association of Official Analytical Chemists
Beta-amylase	An enzyme that degrades starch from the non-reducing end
BGN	Bambara groundnut
BGN-BM	Bambara groundnut base malt
BMS	Base malt syrup
BGN-CM	Bambara groundnut caramel malt
CMS	Caramel malt syrup
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
FAME	Fatty acid methyl esters
FCR	Folin–Ciocalteu reagent
FRAP	Ferric ion reducing antioxidant power
GC-FID	Gas chromatography-flame ionisation detector
GC-MS	Gas chromatography-mass spectrometry
IBM SPSS	International Business Machines Corporation Statistical Package for the Social Sciences
K-BETA3	β-Amylase Assay Kit (Betamyl-3)
K-CERA	α-Amylase Assay Kit (Ceralpha Method)
Kilning	The final stage of malting, after steeping and germinating
MANOVA	Multivariate Analysis of Variance
MRPs	Maillard reaction products
Okpa	A traditional Nigerian food that is very popular in the eastern parts of Nigeria made with Bambara flour
BGN-RM	Bambara groundnut roasted malt
RMS	Roasted malt syrup
Speciality malt	Malts that are used to enhance colour and flavour
BGN-TM	Bambara groundnut toasted malt
TMS	Toasted malt syrup

CHAPTER ONE
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Malting is the germination of seed grains in a controlled environment, followed by immediate termination of the germination process by applying a heat process called kilning to dry the grain (Baranwal, 2017). The three major steps of the malting process are soaking, sprouting (germination) and drying (kilning) (Mäkinen & Arendt, 2015). Two important enzymes, α-amylase, which degrades starch and β-amylase, which attacks the non-reducing ends of the dextrin chains, are activated during the malting process (MacLeod & Evans, 2016). Malts are mostly produced from barley, but it has also been produced from other cereals, pseudo-cereals and legumes (Onwurafor *et al.*, 2020; Ikram *et al.*, 2021).

Different malts are produced for different purposes, including the desired food or beverage products with specific characteristics including colour, texture, flavour, and nutritional enrichment. These include base malts produced from green malt (wet sprouted grain) kilned at low temperature (50-70^{o}C) short duration to give the lightest colour with a grainy flavour; caramel malts produced from the green malt, roasted at a higher temperature (66-70^{o}C) than the base malts; toasted malts made by kilning dried base malts at 168-200^{o}C; and roasted malts produced from green malt by first kilning at low temperatures (70^{o}C) to achieve low moisture content then at between 215-250^{o}C to a dark coloured malt (MacLeod & Evans, 2016; Michael, 2014). All these malt types are further processed to produce malt extract for use in food, beverage, pharmaceuticals industries, and homes (Giebel, 2015).

Malt extracts are produced from malts by mashing milled malt in water and held for a specified period at a regulated temperature and then filtered (Mette *et al.*, 2018). The filtered liquid is concentrated by heating until it turns into a syrupy consistency, which can then be spray-dried to powder (Mette *et al.*, 2018). Malts and malt extracts (liquid and dry malt extracts) can be used for a variety of food products, including pre-digested foods for invalids or infants, malt coffee, sweets, biscuits, bakery products, breakfast cereals, sauces, soft drinks, beers, wines, and malted beverages (Baranwal & Sankhla, 2019).

Due to the urgent need for additional plant foods to meet the nutritional requirements for the developing countries and the world, legumes such as mung beans, soybeans , cowpea and Bambara groundnut seeds have been malted for their functionalities in food production. It has been reported that malting increases enzyme activities, improves

nutritional quality, reduces anti-nutritional contents, enhances flavour and increases amino acids in legumes (Manickavasagan & Thirunathan, 2020).

Bambara groundnut (*Vigna subterranea* (L.) Verdc) [BGN] is a hardy, drought-resistant legume crop cultivated mainly by rural farmers (Hardy & Jideani, 2016; Mayes *et al.*, 2019). The drought-resilient (avoidance, escape, and tolerance) and nutritional characteristics (18–24% protein, 51–70% carbohydrate, 4–12% crude oil, 3–5% ash and 3–12% fibre) of BGN have made it to be described as a leguminous crop for global food security and nutrition (Tan *et al.*, 2020; Khan *et al.*, 2021). Various processing methods have been applied to encourage BGN use (Mubaiwa *et al.*, 2017). Some of the methods that are applied to encourage the use of BGN are cooking with alkaline salts, milling, roasting, fermentation, and malting (Mubaiwa *et al.*, 2017).

Malting of BGN increases seed flour yield due to the change in the cell wall structure, making the seed friable, and decreasing the milling energy (Mubaiwa, 2018). Malting BGN also improved and enhanced its physicochemical characteristics (Nzelu, 2016). High energy, low viscosity weaning and composite foods have also been produced from the pre-digested malted BGN seeds (Hillocks *et al.*, 2012; Ibrahin & Ogunwusi, 2016). In addition, studies have shown that steeping and sprouting encouraged metabolic processes that lead to different alterations in metabolite levels resulting in improved nutrient concentration (Shu *et al.*, 2008; Frank *et al.*, 2011; Lemmens *et al.*, 2019). Due to the dynamic changes during malting, metabolite profiling is an appropriate tool to quantify and follow the dynamic changes during the malting process of cereals and legumes (Frank *et al.*, 2011; Farag *et al.*, 2021a,b; Ibrahim *et al.*, 2021). Therefore, the changes in metabolite levels of cereals and legumes have been studied by metabolomics tools, including gas chromatography-flame ionization detection (GC-FID), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) (Shu *et al.*, 2008; Frank *et al.*, 2011; Jom *et al.*, 2011; Ibrahim *et al.*, 2021).

Sprouted BGN with improved physicochemical, nutritional, functional, thermal properties, and *in vitro* digestibility has been used as a composite ingredient in food formulation (Adeleke *et al.*, 2017; Afolabi *et al.*, 2018; Chinma *et al.*, 2021). The malted BGN could also be further processed into an extract that could be used in a similar popular barley malt extract as an ingredient in food formulation. Considering the metabolic activities that occur during malting, BGN could help combat micro-and macro-nutrient deficiencies common in developing countries. Furthermore, an increase in the abundant non-essential glutamic acids of malted BGN seed could be an advantage because glutamic acid is a flavour enhancer that could increase malted BGN extract as a food processing aid in the food industry (Abba *et al.*, 2018; Alabi *et al.*, 2020; Hellwig and Henle, 2020).

1.2 Statement of the Research Problem

Bambara groundnut is still underutilised despite research carried out in different parts of the world. The acceptability of BGN will be based on its easy accessibility without having to go through the rigours of processing at home. Uvere *et al.* (1999) significantly improved the flour yield of BGN by germinating BGN seeds over five days and kilned dried at 50°C before milling. The effects of malting on the milling performance and acceptability of BGN seeds and their products showed that malting for 1–2 days and drying at 50^{o}C gave an improved flour extraction yield. Furthermore, analysis of the effect of germination and fermentation on the nutritional quality of BGN and its milk product was carried out by Obizoba & Egbuna (1992), where it was observed that germination decreased their anti-nutritional factors. Malting has been shown to effectively reduce anti-nutritional factors and improve the nutritional value of BGN seeds (Adeleke *et al.*, 2017). It has also been shown that germinated BGN flour performed very well in the preparation of *okpa* (Nigerian local steamed gel food). Malting of grains, including BGN, has shown benefits, including ease of milling due to their brittleness, enhanced enzyme activity, nutrient content, increased fibre, flavour production, diverse colour, and reduction in anti-nutrients (Lekjing & Venkatachalam, 2020; Pilco-Quesada *et al.*, 2020). Due to these benefits, malted grains and legumes have been used to develop nutritious foods for different objectives, especially as infants foods, because of their ability to be readily digestible (Harasym *et al.*, 2020). The malted and un-malted BGN seeds have been subjected to phytochemical, nutritional, biological studies and the establishment of their potentials to combat global food insecurity due to their high nutritional values (Khan *et al.*, 2021). The particle size distribution of the BGN flours, paste viscosity and organoleptic properties were acceptable with a slightly coarse particle size after three milling passes (Barimalaa *et al.*, 2005). Also, targeted profiling of phenolic compounds in sprouted BGN has been carried out by Nyau *et al.* (2017). The study used High-Performance Liquid Chromatography-Photo Diode Array-Electrospray Ionization-Mass Spectrometry (HPLC-PDA-ESI- MS) and Folin-Ciocâlteu assay, and an increase in total polyphenol content up to 1.3-fold was recorded. However, nothing has been done on the metabolite profiling of the BGN malting process and the physicochemical characteristics of its malt extracts. While the use of BGN malts and their extracts will be largely dependent on the utilisation of amino acids released during malting, it will be of health benefits to consumers and be convenient and easy to access its nutritional and therapeutic qualities. Thus, there is a need to investigate the metabolite profile of the BGN malting process and the biochemical and physicochemical characteristics of its malt extracts.

1.3 Objectives

1.3.1 Broad objective of the research

The broad objective of the study is to profile the metabolites of the Bambara groundnut malting process and the characteristics of its malt extracts.

1.3.2 Specific Objectives

The research specific objectives include to:

1. Establish the malted BGN seeds with optimum amylase enzyme activities.
2. Determine the effect of the malting process on BGN metabolites.
3. Establish the physicochemical and biochemical characteristics of BGN speciality malt and syrups obtained from the optimum BGN malt.

1.4 Hypotheses

It is hypothesised that:

1. The malting process will significantly affect the amylase enzyme activity of BGN seeds.
2. The malting process will affect the BGN metabolites.
3. The BGN malts and syrups produced from optimum amylase will exhibit good physicochemical and biochemical characteristics.

1.5 Delineation of the Research

The BGN seeds used in this study were used as purchased from Mpumalanga Province in South Africa without sorting into colours.

1.6 Significance of the Research Study

In food science, studies have shown that food products consist of metabolites as affected by processing or pre-processing methods (Cevallos-Cevallos and Reyes-De-Corcuera, 2012). Extensively, food science is emerging in metabolomics, coined foodomics, which has been applied to food to gain insight into the metabolic activities of food before, during and after processing to understand the relationship between food and its health benefits for consumers (Álvarez-Rivera et al., 2021). Metabolic changes during malting make it an important food processing method; it initiates digestive enzymes biosynthesis and encourages changes in phenolic and antioxidant activities during the soaking and sprouting stage (Nemzer et al., 2019). The product from the malting process, malt, is a natural food product used as ingredients in spirits, vinegar, bread, breakfast cereals, cosmetics, and medicine due to its improved nutrient availability. This research will produce new knowledge on the metabolites in the sprouted BGN seeds and malt products that will open an avenue

of new product development for the country and health-conscious individuals. It will also serve as a new way of utilizing the under-utilized legume.

The malt products and their malt extract syrups can be used as condiments or ingredients in food production due to the high glutamic acid, a non-essential amino acid higher in sprouted BGN, which is responsible for the umami taste (Thuy et al., 2020; Wang et al., 2020). Therefore, this study will offer a convenient way to access BGN nutritional content and economically empower women who predominantly farm BGN, improve their lives and families, and promote gender equality. Furthermore, cultivation and production of BGN in large quantities will be encouraged, leading to food security, sustainability, and exportation of the legume to generate wealth and jobs in the nation. Furthermore, the cultivation of BGN in large quantities will contribute to reduce extreme hunger with improved nutrition and sustainable agriculture promotion.

Lastly, it is presumed this study will contribute to the existing body of knowledge on the importance of BGN, leading to new openings for research in South Africa on the use of the underutilized crops in the country, encouraging partnerships for research. On completing this study, a Master's degree will be produced, which will increase the postgraduate output of the Cape Peninsula University of Technology Institute and provide education on the potentials of BGN, resulting in innovation that will encourage partnerships for research nationally and internationally.

1.7 Expected Outcomes, Results, and Contribution of the Research

It is expected that BGN malts and their malts extracts syrup will be produced. The physicochemical and metabolites characteristics of BGN malt and extract will be established. New knowledge of the metabolites of the BGN malts will be established. As a result, new products with high nutritional constituents that will add to the daily intake of protein for consumers will be produced to alleviate malnutrition, undernutrition, and over-nutrition in South Africa.

A Master thesis will be produced from this research study. At least one article will be published in an accredited international journal, and the research output will be presented in at least one national or international conference.

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, the significance of the research, expected outcomes, results, and contributions of the research. Chapter two is the literature review based on the BGN

background, including its nutritional composition, various uses, potential use, and prospects. Next, the BGN processing methods, physicochemical characteristics and nutritional benefits were discussed. Finally, the malting process, metabolomics, and importance and potentials of BGN in the food industries were reviewed.

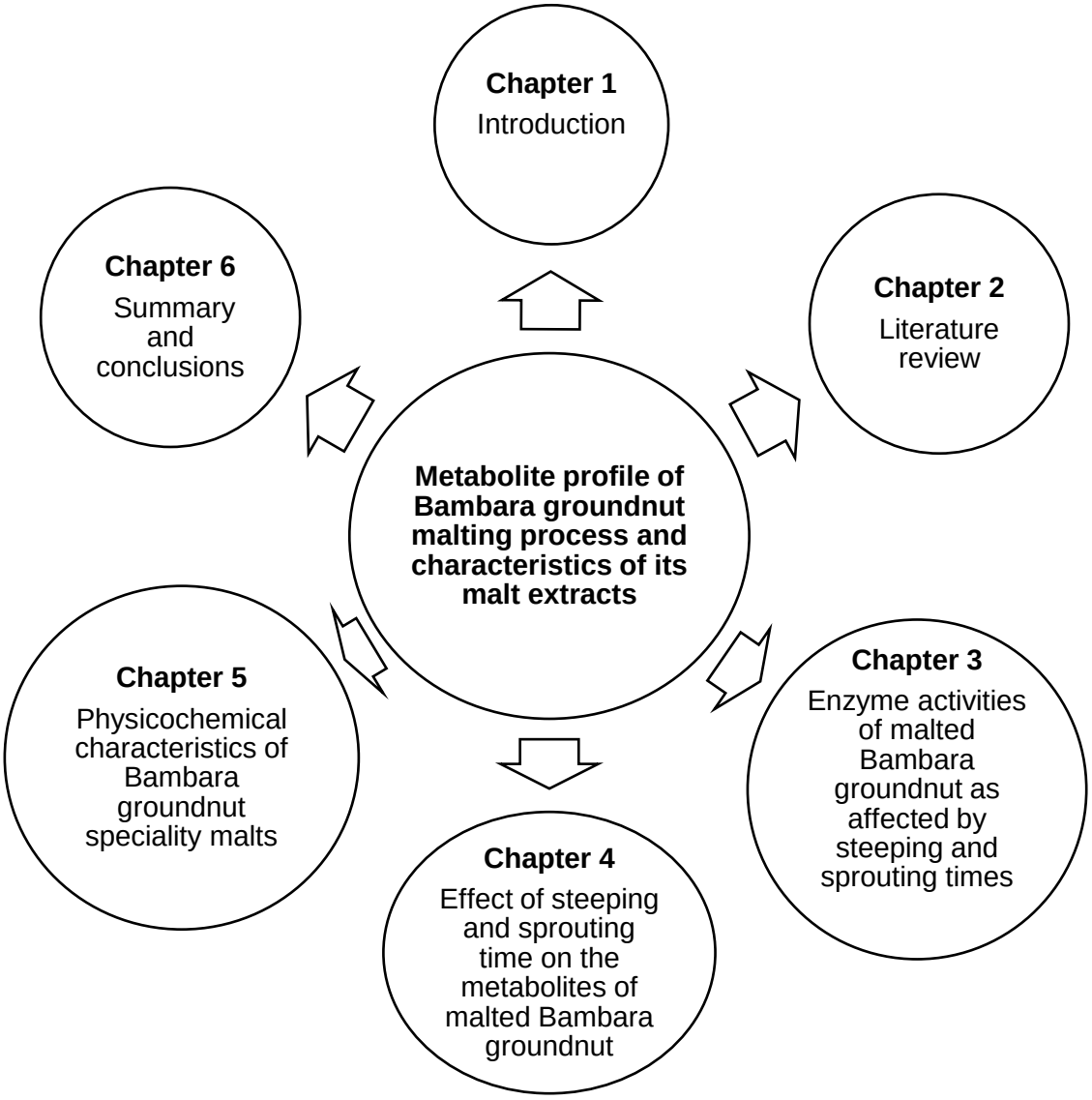

Figure 1.1: Structure of the thesis

Chapter three is the first research chapter focusing on the malting process of BGN. The physicochemical properties, polyphenolic, antioxidants, and amylase activities of malted BGN. The physicochemical, polyphenolic, antioxidants and amylase activities based on steeping and sprouting time were discussed. Chapter four is the second research

chapter focusing on the production of BGN malts and their metabolite characteristics. They were profiled for fatty acid methyl esters, hydrocarbons, sugar alcohols, sugars, organic acids, and amino acids. Chapter five is the third research chapter focusing on the production of the speciality malts and their malt extract syrups (MES). The speciality malts and syrups physicochemical and metabolite characteristics were determined and discussed. The speciality BGN malts were also profiled for fatty acid methyl esters, hydrocarbons, sugar alcohols, sugars, acids, and amino acids. Chapter six summarizes and concludes the research and offers recommendations for future studies.

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CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Malting

Malting is seed germination under strictly controlled environmental conditions to give a desirable physical and biochemical change within the grain, which is then stabilised by drying the grain to give the final product called malt (Rani & Bhardwaj, 2021). The malting process is a simple, low-cost household food processing applied to cereals and legumes to improve their physicochemical properties by biochemical modifications (Hejazi & Orsat, 2016). It comprises three main steps: steeping, germination (sprouting) and kilning. The steeping step allows the grain to take up water up to 43–46% for stimulation of germination (Ofoedu *et al.*, 2021). During the 4 to 7 days of germination (sprouting) period, the grains (cereals and legumes) produce hydrolytic enzymes, which lead to modifications of the grain structure then germination is terminated by kilning to reduce the moisture, thus, stopping metabolic activity (Gupta *et al.*, 2010; Alu'datt *et al.*, 2019; Rani & Bhardwaj, 2021). In addition, the metabolic hydrolytic enzyme activities allow the production of low molecular weight compounds like simple sugars that contribute to the Maillard reaction (Mosher & Trantham, 2021).

The germination process is vital because it produces the hydrolytic enzymes (Aubert *et al.*, 2018). The hydrolytic enzymes are classified as cell wall hydrolases (arabinoxylanase and β-glucanase); starch hydrolases (α-amylase, β-amylase, limit dextrinase and α-glucosidase} and proteolytic enzymes (Rani & Bhardwaj, 2021). The synthesis of gibberellic acid (GA), a growth-promoting hormone in the grain embryo during steeping, encourages diffusion through the grain to initiate the production of the hydrolytic enzymes (Ali & Elozeiri, 2017). The large complex compounds starch and proteins present in grain are degraded into simpler forms during germination by the hydrolytic enzymes. During malting, α-and β-amylases are the major enzymes hydrolysing starch into simple fermentable sugars (Rani & Bhardwaj, 2021). Alpha-amylase is an endo-enzyme that hydrolyses long-chain saccharides α-1,4-glycosidic bonds and belongs to the glycoside hydrolases family (Iskakova *et al.*, 2017; Rani & Bhardwaj, 2021). β-Amylase is an exo-amylase that hydrolyses maltose from the non-reducing ends of amylopectin near the α-1→6-D-glucosidic bond (Gupta *et al.*, 2010; Rani & Bhardwaj, 2021). However, these enzymes are heat sensitive and are easily denatured at high temperatures; thus, the kilning temperatures must be well regulated. For example, α-amylase can be denatured at 65^{o}C, while β-Amylase can be rapidly denatured at temperatures above 55^{o}C (Ferrari-John *et al.*, 2017). The end product, malt, is desired by the food industries based on its characteristic properties, including the enzymes, aroma, texture, and flavour.

Malt is produced mostly from barley, but other cereals like wheat, rye, oats, millet, sorghum and legumes have been malted (Mulder, 2005; Bekele *et al.*, 2012; Ding & Feng, 2019). Malt is one of the main ingredients used in beer production, as a source of starch and contributing to the colour and organoleptic characteristics of beer (Edney & Izydorczyk, 2003). It is used for brewing because it contains starch and enzymes to convert into sugars, proteins for the body, foam and yeast nutrition, and plenty of great flavours to delight the consumers (Green *et al.*, 2020). While most of the malts produced are used in the brewing and distillation industries, malt can be used in many other food products (Green *et al.*, 2020). These products range from baked products, breakfast cereals, non-alcoholic beverages such as malt drinks (Hübner & Arendt, 2013; Kaushik *et al.*, 2017; Alu'datt *et al.*, 2019). Malted grains are used in food products to enhance colour, flavour, improve nutrients and taste. These have been recorded to be used effectively in weaning foods (Abiose *et al.*, 2015; Augad & Wankhade, 2016), bread and biscuit (Ayo *et al.*, 2014), and improvement in the nutritional content of grains (Kaushik *et al.*, 2010; Carciochi & Manrique, 2014; Mäkinen & Arendt, 2015).

Miyahira *et al.* (2021) documented some scientific studies on the use of sprouted grains and legumes to develop new food products and their effects. Tables 2.1 and 2.2 show the effect of sprouted seeds, cereals, pseudo-cereals, and legumes on food products. Amaranth and chia seeds exhibited an increase in the concentrations of dietary fibre, soluble protein, total phenolic content, total flavonoid content, total anthocyanin content, antioxidant activity and high sensory acceptability (Perales-Sánchez *et al.*, 2014; Sandoval-Sicairos *et al.*, 2020). Gómez-Favela *et al.* (2017) also noted higher protein, total phenolic, antioxidant activity, γ-aminobutyric acid, essential amino acids, and total dietary fibre contents in sprouted chia seed flour. The sprouted chia seed flour improved characteristics make it a beneficial functional ingredient in new food formulation. Blue maize, brown rice, and buckwheat germinated to be used as an ingredient in food formulation and fortified bread showed promising bioactive components that could be of benefit to consumers (Alvarez-Jubete *et al.*, 2010; Argüelles-López *et al.*, 2018; Chavarín-Martínez *et al.*, 2019). The blue maize had increased protein content, antioxidant activity, and total phenolic, dietary fibre, and anthocyanin (Chavarín-Martínez *et al.*, 2019). Bread made from germinated buckwheat flour contained a significant number of flavonoids (Alvarez-Jubete *et al.*, 2010). The formulated bread with a ratio of 70:30 of germinated brown rice to wheat flour showed higher alpha-amylase activity than only wheat flour (Charoenthaikij *et al.*, 2010). Also, there was no significant difference in the acceptability of the bread from germinated brown rice for aroma, flavour, and taste compared to the control wheat bread.

Table 2.1 Effect of sprouted seeds, cereals, and pseudo-cereals on food products

Sprouted seeds	Sprouting time	Food products	Beneficial effect	Reference
Amaranth	78 h	Sprouted amaranth	Increase in soluble protein concentrations, total phenolic content, and antioxidant activity.	Sandoval-Sicairos *et al.* (2020)
	14-120 h		Increase in antioxidant activity, total phenolic and flavonoid contents. Decrease in total lipid content.	Perales-Sánchez *et al.* (2014)
Amaranth and chia	78 h	Amaranth and chia flour beverages	Increase in protein and dietary fibre contents and high sensory acceptability.	Argüelles-López *et al.* (2018)
Blue maize	12–220 h	Sprouted blue maize flour	Increased protein content, antioxidant activity, and total phenolic	(Chavarín-Martínez *et al.* (2019)
Brown Rice	24-48 h	Sprouted brown rice flour Bread	Formulation of 70:30 with germinated brown rice had higher alpha-amylase activity	Charoenthaikij *et al.* (2010)
Buckwheat	96 h	sprouted buckwheat flour bread	Bread made using contained flavonoids in significant amounts.	Alvarez-Jubete *et al.* (2010)
Chia	10–300 h	Sprouted chia flour	Higher protein and total phenolic contents than non-sprouted chia flour	Gómez-Favela *et al.* (2017)

Table 2.1 Effect of sprouted seeds, cereals and pseudo-cereals on food products

Sprouted seeds	Sprouting time	Food products	Beneficial effect	Reference
Quinoa	12-72 h	Sprouted quinoa flour	Improved the stability of the foam, increasing amylolytic enzyme levels.	Suárez-Estrella *et al.*, (2020)
Sorghum	48 h	Sprouted sorghum flour	Reduction of anti-nutritional factors	Ojha *et al.* (2018)
Wheat	5 days	Sprouted wheat flour	Increased tocopherols, niacin, riboflavin, and free and bound phenolic compounds.	Žilić *et al.* (2014)
	6 days		Gluten degradation is promoted by germination.	Boukid *et al.* (2018)
	4 days		Increased phenolics and protein contents.	Świeca *et al.* (2017)
	4 days		Increase in antiradical and chelating compounds as well as phytochemicals.	Gawlik-Dziki *et al.* (2017)
	10 h	Wheat bread enriched with sprouted wheat flour	Baking properties are comparable to those of control flour	Tian *et al.* (2019)
Barley	3 days	sprouted barley flour bread	Increased in peptides, free amino acids, and γ-aminobutyric acid contents. High digestibility protein content in bread	Montemurro *et al.* (2019)

Table 2.2 Effect of sprouting on legume food products

Sprouted seeds	Sprouting time	Food products	Beneficial effect	Reference
Lentil	5 days	Sprouted lentil flour	Increase in phenols and flavonoids in 100% lentil sprouted flour bread with sensory acceptance	Hernandez-Aguilar *et al.* (2020)
Lentil and chickpea	5 days	Lentil and chickpea flour	Increase in peptides, free amino acids, and γ-aminobutyric acid contents. Decreased concentrations of phytic acid, condensed tannins, raffinose, and trypsin inhibitors. Bread: high protein digestibility. specific volume, not significant differences	Montemurro *et al.* (2019)
Moth bean	48 h	Sprouted moth bean flour	Higher gelation, thermal stability and lower viscosity due to degradation compared to non-sprouted beans.	Medhe *et al.* (2019)
Mung Bean	36 h	Composite flour Bread	Phenolic and protein contents increase	Menon *et al.* (2015)
	72 h	Sprouted mung bean flour	Protein content and functional properties improvement (water absorption, water solubility, oil absorption ability, and water retention).	Liu *et al.* (2018a)
Bambara groundnut	72 h	Sprouted Bambara flour	Increases in protein, dietary fibre components, digestible starch, in vitro protein digestibility, calcium, magnesium, potassium, total phenolic content, and amino acids. Decreased phytic acid, tannin and trypsin inhibitory activity. Increase protein solubility, oil and water absorption capacity, foaming capacity and emulsion stability. Modification of the thermal and pasting properties of the flour	Chinma *et al.* (2021)

Sprouted lentil, moth bean and mung bean were used as an ingredient in bread and composite noodles production (Liu *et al.*, 2018a; Medhe *et al.*, 2019; Hernandez-Aguilar *et al.*, 2020). Bread made with 10% sprouted lentil flour showed an increase in the content of phenols and flavonoids with acceptable sensory quality. However, it was harder and had less cohesiveness than wheat bread, which was attributed to the greater resistance of the swollen starch during the cooking process. Sprouted moth beans exhibited high gelation, thermal stability, and low viscosity degradation compared to non-sprouted moth beans (Medhe *et al.*, 2019). However, a decrease in ash content was attributed to the draining of macro-and micro-elements during soaking. Sorghum, wheat, barley, chickpea, and quinoa grains studied for their use as ingredients in food formulations and composite bread showed improved bioactive compounds. Sprouted sorghum flour showed a reduction in anti-nutritional factors such as phytate, tannin, oxalate, and improved functional properties (Ojha *et al.*, 2018). Sprouted wheat used as an ingredient in the formulation of food products had an increase in the levels of tocopherols, niacin, riboflavin and phenolic compounds (free and bound) (Žilić *et al.*, 2014). The baking properties of sprouted wheat were good compared to those of control flour, with gluten degradation promoted by the germination process with increased phenolics and protein contents (Gawlik-Dziki *et al.*, 2017). The study also showed that there was an increase in antiradical, chelating compounds, and phytochemicals, which made the bioactive compounds bio-accessible. However, Tian et al. (2019) noted a decrease in total phenolic content, flavonoid content, and antioxidant activity in wheat bread enriched with sprouted wheat.

Sprouted chickpea and quinoa grains flour were reported to increase peptides, free amino acids, and γ-aminobutyric acid contents, decreased concentrations of phytic acid, condensed tannin's raffinose, and trypsin inhibitors. Meanwhile, the composite bread had highly digestible protein, but there was increased microbiological contamination of the flour, and the bread was hard and fracturable (Montemurro *et al.*, 2019). On the other hand, the sprouted cereals, pseudo-cereals, and legumes all exhibited improved biochemical properties, which are the properties malt users look out for when using malt as ingredients in food production.

Malts are used due to their improved biochemical properties, during which proteases break down cellular proteins and enzymes to get free amino acids (Avilés-Gaxiola *et al.*, 2018). Singh *et al.* (2015) noted that the nutritional and sensory properties of cereals and pseudo-cereals could be enhanced by malting, which leads to improved product properties. It was observed that the increased nutritional value and better digestibility of the grains made them better food material than the raw grains. In addition, when subjected to germination, legume seed grains nutritional composition was improved, whereby their protein content increased (Liu *et al.*, 2018; Wu & Xu, 2019). Malted grains have also been reported to have increased concentrations of vitamins, trace elements and minerals bioavailability (Ikram *et al.*, 2021).

Therefore, the malt produced from the cereals and legumes can be used for ready-to-eat food and beverage products that consumers can easily digest (Wu & Xu, 2019). However, most of the physical and aromatic characteristics of malted grains are contributed by kilning.

The aim of kilning is to prevent more roots and growth, to reduce starch modification, to attain a stable malt product for storage and transportation to other areas, to save the enzymatic activity, and for development and stabilisation of colour, aroma and flavour (Schwarz & Li, 2010; Hill, 2015; Bamforth, 2017). The process also eliminates unwanted flavours and chemical compounds (Bamforth, 2017; Green *et al.*, 2020). In addition, the malt is friable after kilning and can be ground for brewing processing or stored in dry conditions for up to 12 months without any loss of quality (Green *et al.*, 2020).

The kilning temperature also brings about Maillard reactions which always lead to producing different types of colour and aroma compounds (Kleinwächter *et al.*, 2014; Singh *et al.*, 2015; MacLeod & Evans, 2016). Mostly, drying is done in three phases where the first drying period is about 24 hours 50^{o}C temperature. The second drying period is 12 hours, at a slightly higher temperature around 65-75^{o}C. The third and final drying period, the curing process (moisture content reduced to less than 6%), will be over the next 24-48 hours at 83-104^{o}C (Briggs, 1998; MacLeod & Evans, 2016; Rani & Bhardwaj, 2021). The curing process affects the malt's simple sugars and amino acids by their combination in the Maillard reaction resulting in colour, taste, aroma, stability and longer shelf life (Skendi & Papageorgiou, 2018). Therefore, the malt style determines the temperature and length of the kilning and the roasting process to follow, as listed in Table 2.3 (Davies, 2006, 2016; Puremalt, 2018; Skendi & Papageorgiou, 2018).

Table 2.3 Types of speciality malts

Kilning process	**Kilning temperature(^{o}C)**	**Kilning time (h)**	**Malt type**
Green malt kiln-dried	98-105	22-24	Vienna, munich, al pilsner
Green malt roasted	100-140	2-3	Amber, Caramalt, crystal
Dried kiln malt roasted	200-228	2-3	chocolate, black
Raw barley roasted	228-250	1-2	Roasted barley

Malt is therefore kilned to produce a friable, readily milled, stable product that may be stored for a long time (Guido & Moreira, 2013). Also, kilning conditions are fundamental to developing high-quality malts from cereal and legume grains (Augad & Wankhade, 2016).

Kilning conditions significantly contribute to the final malt behaviour; therefore, careful control is applied to avoid losing heat-sensitive beneficial food components of the grains. Furthermore, the kilning stage allows a range of speciality malt types to be produced (MacLeod & Evans, 2016). Since the conditions of kilning contribute to the final malt physicochemical characteristics; thus, kilning is controlled to allow a range of speciality malt varieties to be produced. Green malt kiln-dried consist of Vienna, Munich, ale and pilsner malt types and are dried between 98 and 105^{o}C for 24 h, green malt roasted (Amber, Caramalt, crystal) is dried at 100-140^{o}C for 2 to 3 h. Chocolate and black malts are categorised as the dried kiln malt roasted at 200-228^{o}C for 2-3 h while the raw barley roasted malt is dried at 228-250^{o}C for 1-2. Malts with a colour range of 2 to 30 ^{o}EBC (European Brewing Convention units) are made in a malt kiln at low temperatures (Pahl *et al.*, 2016). Roasted malts are higher in colour with range 35–1600 ^{o}EBC and are produced from raw barley, malt, or green malt (Coghe *et al.*, 2004). However, due to the high temperature applied to the speciality malts in the drum roasting plants, enzymatic activity is destroyed, but characteristic malts with strong flavours and colours (MacLeod & Evans, 2016). Although most of the malts produced are used in the brewing industries, food processing companies also use them to produce amylase-rich food products to add flavour and colour to their products (MacLeod & Evans, 2016). The malts are also further processed into malt extract syrup or powder for use industrially or domestically (Felix, 2020).

2.1.1 Production and Uses of Malt Extract Syrup

Malt extract syrup is a liquid syrup or powder made by milling and mashing malted cereal grains to produce extracts, and then the clarified, filtered liquid is concentrated (Yousif & Evans, 2020). The malt extract syrup is produced by crushing the malted grains and mashed; then, the wort is separated from spent grains, concentrated, and dehydrated following the brewing process (Hansen, 2008). First, malted grains are mashed at 65-85^{o}C with water to allow the enzymes to digest the starch and protein (Felix, 2020). Once the insoluble fibre (spent grain) is removed, a sugary liquid is produced, then it is concentrated to make a viscous, stable liquid sweetener called malt extract syrup (Felix, 2020). Finally, the concentrated malt extract is categorised into liquid malt extract (LME) with a syrup consistency which can be further dried in the spray dryer to produce a dried malt extract (DME) (Rögner *et al.*, 2021). The subsequent product is packed for further use, either in the syrupy or dried forms in homes, food, brewing, and pharmaceutical industries based on the user's specification (Carroll, 2017; Rosentrater & Evers, 2018).

Malt extract is an essential ingredient for manufacturing good quality food products, as they contain high levels of amylase, antioxidants, vitamins, and proteins (Lee *et al.*, 2010; Qingming *et al.*, 2010; Giebel, 2015; Davies, 2016). Therefore, it is widely used in

the food, brewing, distilling, pharmaceutical, and bakery manufacturing industries (Rögner *et al.*, 2021). For example, it is used to brew various beers, wines, and distilleries (Davies, 2016). It is also used to enhance and improve the flavour, colour and nutrient content in confectionery, breakfast cereals, and baked food products (Giebel, 2015; Fermentation Solutions, 2021). They have also been used to improve the flavour of cereal-based foods such as the ready-to-use infant formula, biscuits, and bread. Ice cream, milkshake and malted beverages also depend on malt extract for their taste, sweetness, and flavour (Weston, 1984; Obatolu, 2002; Davies, 2006; Ayo et al., 2014; Mäkinen & Arendt, 2015; Augad & Wankhade, 2016; Carroll, 2017; Rosentrater & Evers, 2018).

Due to consumers' current awareness of healthier food and reducing sugar use in their daily diets, malt extract has become popular in research and application in new food productions (Maheshwari, 2020; Targan, 2020; Fermentation Solutions, 2021). Additionally, new food innovation is driven mainly by consumer demand, coupled with the fact that many consumers are interested in healthy foods with clean ingredient labels and plant-based origins (Rosentrater & Evers, 2018; Shoup, 2019; Green *et al.*, 2020; Rögner *et al.*, 2021). Hence, malts and malt syrups are used as ingredients and condiments in food and beverage production.

Mostly cereal grain malts and their extracts are used in food processing. Still, malted legumes can also produce extracts in liquid or powdered form to access their nutritional values and overcome laborious household processing. Recent studies reported the use of malted legumes to produce extracts that would be readily acceptable in the food and beverage industries (Black *et al.*, 2018; Gasiński *et al.*, 2021). Gasiński *et al.* (2021) produced congress worts from vetch (*Vicia sativa*), green lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), and yellow pea (*Pisum sativum*) seeds malts. The study reported increased phenolic compounds and antioxidant activities of the malt extract. Bambara groundnut, an ‘underutilised’ legume that originated from Africa, can also be used to make malt extract with high protein content, reduced anti-nutritional content and increased antioxidants components. Bambara groundnut malt, rich in essential and non-essential amino acids like glutamic acid, can be a positive advantage if a liquid or powdered extract is produced similar to soy sauce (Ninomiya, 2015).

2.2 Bambara Groundnut

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) (BGN) is known in different regions of Africa as *izindlubu* (Zulu, South Africa); *jugo beans* (South Africa); *ntoyo cibemba* (Zambia); *gurjiya* or *kwaruru* (Hausa, Nigeria); *okpa* (Igbo, Nigeria); *epa-roro* (Yoruba, Nigeria); *nyimo* beans (Zimbabwe) (FAO, 2018). It originated from West Africa, in the Bambara district near

Timbuktu; hence its name Bambara groundnut (Swanevelder, 1998; Azam-Ali *et al.*, 2001; Jideani & Diedericks, 2014). It is grown mainly by subsistence farmers, predominantly local women in drier parts of sub-Saharan Africa (Jideani & Diedericks, 2014). Local farmers principally cultivate the grain legume as a "famine culture" crop due to its high nutritional value, tolerance to drought and ability to grow on infertile soils, which other crops like cowpea and groundnuts cannot withstand (Gulzar & Minnaar, 2017). In addition, while it is very adaptable to hot temperatures, it can also tolerate excessive rainfall (Swanevelder, 1998). Therefore, it is not at risk of total crop failure (Diedericks *et al.*, 2020a; Jideani & Jideani, 2021). Bambara groundnut is an indigenous grain legume of different colours, as shown in Figure 2.1 (Murevanhema & Jideani, 2013; Muhammad *et al.*, 2020; Ngui *et al.*, 2021).

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Figure 2.1: Bambara groundnut
Source: Jideani (2016)

The ability of BGN to grow in poor soils, nitrogen fixation and drought tolerance, coupled with sufficient quantities of valuable nutrients like protein, carbohydrates and fats, are important features for sustainable crop production (Gulzar & Minnaar, 2017; Ngui *et al.*, 2021). Bambara groundnut is ranked as the third most important grain legume after groundnut and cowpea (Gulu, 2018; Tan *et al.*, 2020; Oyeyinka *et al.*, 2021). It serves as an important protein source in the poor regions of Africa because it is not expensive, especially in a situation whereby poor people cannot afford the costly animal protein (Azman *et al.*, 2019a; Muhammad *et al.*, 2020). It can also be easily stored and transported to rural and urban populations (Baryeh, 2001; Bamshaiye *et al.*, 2011). The seeds of BGN make a complete food, as it contains sufficient carbohydrate (63-65%), protein (16-19%) and fat (6.5%) which makes it a high energy food compared to other legumes (Tan *et al.*, 2020; Khan *et al.*, 2021). The nutritional content and high amounts of essential amino acids (methionine, leucine, isoleucine, lysine, phenylalanine, threonine, valine, and tryptophan) of BGN has made it to be considered a crop for food security. (Gqaleni, 2014; Yao *et al.*, 2015; Khan *et al.*, 2021). Raw BGN seeds are rich in glutamic acid, aspartic acids, leucine and lysine compared to the FAO/WHO recommended daily intake of amino acid as complied by Azman *et al.* (2019a) and shown in Table 2.4.

Table 2.4 Range of amino acid concentrations in Bambara groundnut raw seeds

		FAO/WHO daily intake recommendation (% protein)		
Amino acid	**Concentration (% protein)**	**Infants (0–6 months)**	**Children (6–36 months)**	**Adolescents (> 36 months)**
Histidine	0.59-4.09	2.10	2.00	1.60
Leucine	1.33-10.22	9.60	6.60	6.10
Lysine	0.99-8.54	6.90	5.70	4.80
Threonine	0.61-5.22	4.40	3.10	2.50
Tryptophan	0.12-0.60	1.70	0.85	0.66
Valine	0.71-6.47	5.50	4.30	4.00
Arginine	1.20-8.25	n/a	n/a	n/a
Methionine	0.30-6.41	3.30	2.70	2.30
Cysteine	0.12-2.41			
Aspartic acid	1.94-15.12	n/a	n/a	n/a
Glutamic acid	3.21-21.38	n/a	n/a	n/a

Source: Azman et al. (2019a), n/a Not applicable

International and local researchers have researched to increase BGN utilisation in different ways. However, despite several works that have been done on BGN by researchers, growers, and traders, much still needs to be done to ascertain and access the full potential of the seed grain (Azam-Ali *et al.*, 2001; Azman *et al.*, 2019a). Some of the reasons for the underutilisation could be its difficulty in cooking, strong beany flavour, slightly bitter taste, poor dehulling properties of the seed (Arise *et al.*, 2019). Other reasons for underutilisation are poor milling characteristics and various anti-nutritional factors like tannins oxalate, tannic acid, phytic acid, and phytic phosphorus and trypsin inhibitors in raw BGN seeds (Uvere *et al.*, 1999; Hillocks *et al.*, 2012; Mubaiwa *et al.*, 2017). These anti-nutritional factors reduce the frequent utilisation of BGN by hindering protein digestibility (Azman *et al.*, 2019b; Khan *et al.*, 2021). However, some of the anti-nutritional factors can be reduced to the minimum by subjecting the raw BGN to some domestic food processing such as soaking, roasting, fermentation, treatment with 60% ethanol, germination (sprouting), malting and at times combination of one or two of these processing methods (Alain *et al.*, 2007; Ijarotimi & Esho, 2009; Diedericks *et al.*, 2020; Chinma *et al.*, 2021). Furthermore, the removal and reduction of the anti-nutritional components studied by researchers made BGN an important potential use in food formulations industrially (Unigwe *et al.*, 2018).

2.2.1 Uses of Bambara Groundnut

Bambara groundnut is mainly used for human and animal's consumption. A good-quality protein and other well-balanced nutrients like fats, carbohydrates, and mineral contents make it excellent potential for many food processing applications (Gulzar & Minnaar, 2017). The immature BGN seeds can be eaten fresh, boiled, or grilled. It can also be eaten alone or mixed with maize or immature groundnut. The mature dried seeds can be boiled, roasted, and pounded into flour which can be eaten alone or added to other traditional dishes like plantain and maize (Swanevelder, 1998; Jideani & Diedericks, 2014; Gulzar & Minnaar, 2017; FAO, 2018). It is used to make popular steamed food products known as *okpa* and *akara* in different parts of Nigeria and bread-making in Zambia (Uvere *et al.*, 1999). In Ghana and Zimbabwe, commercial canning of BGN in gravy is a successful industry (Swanevelder, 1998; De Kock, 2013). Incorporating BGN in cereal-based foods has also helped enhance and improve the products ranging from snacks, infant weaning food, and complimentary meals to avert malnutrition and undernutrition (Oyeyinka *et al.*, 2021).

Bambara groundnut has been used to produce weaning, composite and complementary foods and snacks that can alleviate malnutrition and under-nutrition (Nwadi *et al.*, 2020; Oyeyinka *et al.*, 2021). Also, BGN has been used in proportions with other grains, fruits and tubers, including sorghum, fonio (*acha*), millet, maize, groundnut, cowpea, plantain,

banana and cassava, to improve the protein content of the formula (Alain *et al.*, 2007; James *et al.*, 2018a; Nwadi *et al.*, 2020). Although Infants and young children feeding are mostly considered when formulating complimentary snacks and meals by researchers, adults too can benefit from BGN supplementation due to its high protein content (Nwadi *et al.*, 2020).

Snacks have always been a relief to the busy schedule of the world population but having a nutritious snack is now more important to the dynamic world due to many diseases and discomfort from junk eaten. In recent times, Oyeyinka *et al.* (2018) concluded that a deep-fried snack named *akara* Ogbomosho produced from cowpea could be produced from BGN flour and paste. The study showed that the sensory properties of snacks made from the paste were superior to that made from the flour in aroma, colour, crunchiness, and overall acceptability. Quality evaluation of snacks produced from blends of unripe plantain, BGN and turmeric with different ratios of BGN formulated in the range of 3 to 100% had improved protein content from 4.23 to 14.81% (Adegunwa *et al.*, 2017). Doughnut, a wheat-based snack, was produced by substituting wheat flour with BGN flour by Adebayo-Oyetoro *et al.* (2017) from blends of wheat and BGN composite flour mixed at ratios (90:10, 80:20, 70:30, and 0:100). An increase in BGN content increased the protein content, but the 10% BGN flour blend was most acceptable. Sudanese *kissra* bread was produced by mixing sorghum and BGN [10%, 20%, and 30%] (Abdualrahman *et al.*, 2019). The protein levels of the *kissra* bread changed at the increase in BGN flour supplementation. Due to the importance of snacking in the world, researchers have gone ahead to look for various ways to utilise BGN for enhancement, fortification and functional properties by the development of new products to meet up with the demand (Akande and Fabiyi, 2010; Abu-Salem and Abou-Arab, 2011; Adebayo-Oyetoro *et al.*, 2017; Adegunwa *et al.*, 2017; Talabi *et al.*, 2019; Agu *et al.*, 2020).

Bambara groundnut milk (liquid and powdered milk) has also been shown to contain all the goodness in the seeds, which may add value to the nutritional well-being of people. (Murevanhema & Jideani, 2013, 2015; George & Awopetu, 2017; Hardy & Jideani, 2018). Extruded meat analogues or textured vegetable proteins made from BGN flour and blends from BGN flour, protein isolate and fermented African breadfruit flour have shown high consumer acceptability. Bambara groundnut can be an effective source of inexpensive protein to replace some of the meat protein in the diet and replace ground meat in various recipes (Charles, 2010; Adedokun *et al.*, 2017). It also contains minimal fat and sodium and can provide the dietary fibre necessary for the human diet (Alakali *et al.*, 2010; Charles, 2010; Adedokun *et al.*, 2017). Bambara groundnut milk produced by Murevanhema & Jideani (2015), Okudu *et al.* (2016) and George & Awopetu (2017) was more acceptable compared to milk from cowpea and soybean. Also, Hardy & Jideani (2019) produced protein-rich BGN milk powder using spray drying. The BGN milk powder has a longer shelf life and can be used in food formulation with lower protein content.

Products such as fufu, a fermented staple food made from cassava, were fortified with BGN by Olapade *et al.* (2014), supplementing with 10%, 20%, 30%, 40%, and 50% of BGN portions which led to increased protein content up to 18.87%. Adebiyi *et al.* (2021) and Ademiluyi & Oboh (2011) produced *dawadawa*, fermented condiments common in Nigeria from BGN seed. The characteristic metabolites, antioxidants and phenolic composition showed that the derived *dawadawa* might serve as functional foods and benefit health. Bintu *et al.* (2015) and (Adeyemo & Abimbola, 2019) produced a complementary weaning food for infants, *ogi/ akamu* (a common fermented slurries made from fermented maize, sorghum or millet in Nigeria) by complementing with BGN seeds. The product showed good physicochemical characteristics and improved nutritional composition required for a growing infant. The studies also noted that fortification of sorghum and maize ogi flour with BGN could reduce protein malnutrition.

The world need for proteins is increasing; therefore, there is a need for food protein from the old and new protein sources. Since all proteins have good nutritional value, studies have been carried out on BGN protein isolates and concentrate (Adebowale *et al.*, 2011; Arise *et al.*, 2017; Alabi *et al.*, 2020; Ngui *et al.*, 2021). It has met the required functional characteristics, exhibiting high water and oil absorption capacities. Furthermore, studies have suggested that the isolates from BGN flour have the potential for usefulness in flavour retention, improvement of palatability, and extension of shelf life in meat products with good foaming capacity and stability (Diedericks *et al.*, 2020; Ngui *et al.*, 2021; Yang *et al.*, 2022). These studies further noted that high emulsifying activity and stability of the BGN isolates has also indicated that they could be used as ingredients in many food formulations, such as salad dressing, comminuted meats, ice creams, cake batters, and mayonnaise (Adebowale *et al.*, 2011; Arise *et al.*, 2015; Adeleke *et al.*, 2018; Yang *et al.*, 2022). Although BGN has potentials for industrial use, the use of BGN is greatly dependent on its processing method due to its hard-to-cook characteristics (Mubaiwa *et al.*, 2018a; Azman *et al.*, 2019b).

The studies and researches carried out over the years on BGN have been channelled to identify BGN industrial potentials for processing and utilisation (Ibrahin & Ogunwusi, 2016; Peduruhewa *et al.*, 2021). However, its utilization is greatly dependent on the processing methods that would not take away its nutritional properties and the products developed, as listed in Table 2.5. Also, processing methods such as milling, dehulling, fermentation, soaking and boiling and malting have been used to increase its utilization (Olagunju *et al.*, 2018; Adebiyi *et al.*, 2019, 2021; Arise *et al.*, 2020; Ezeibe & Asumugha, 2021; Olagunju & Ijabadeniyi, 2021).

Table 2.5 Recent Bambara groundnut food products

BGN product formulation	Product name	Product type and use	References
BGN	BGN milk	Beverage	Okudu *et al.* (2016) George & Awopetu (2017)
	BGN yoghurts	Snack	Falade *et al.* (2015)
	BGN Powdered yoghurt	Bambara groundnut powdered yoghurt	Hardy & Jideani (2019)
	BGN d*awadawa*	Condiment	Adebiyi *et al.* (2021)Ademiluyi & Oboh (2011)
	BGN flour	Ingredient	Nzelu (2016)
	Protein isolates and concentrates	ingredients	Ngui *et al.* (2021) Adebowale *et al.* (2011), Arise *et al.* (2017), Alabi *et al.* (2020)
	Dietary fibre	Thickening agents, stabilisers, ingredients	Maphosa & Jideani (2016)
	Meat	Analogue Meat	Charles (2010)Adedokun *et al.* (2017)
Acha (fonio), malted BGN and date palm flour	Biscuit	Snack	Agu *et al.* (2020)
BGN flour, wheat flour	Biscuit	Snack	Abu-Salem & Abou-Arab (2011)
Yellow maize and white maize cowpea, BGN, and groundnut	*Akamu* (fermented porridge)	weaning food	Bintu *et al.* (2015)

Table 2.5 Recent Bambara groundnut food products continued

BGN product formulation	Product name	Product type and use	References
BGN, sorghum	*Akamu, ogi* (fermented porridge)	Weaning food	Adeyemo & Abimbola (2019)
Cassava-Bambara	*Fufu* flour	composite flours	Olapade *et al.* (2014)
Maize-Bambara nut malt	n/a	Complimentary food	Nwadi & Uvere (2019)
			Mbata & Adeyemo (2020)
BGN, bean, Moringa Seed Flour blend and wheat	Biscuit	Snack	Talabi *et al.* (2019)
Wheat, cassava, and BGN flours	Pasta	Complimentary meal	James *et al.* (2018b)
Acha, BGN flour	Bread	Complimentary meal	Chinma *et al.* (2016)
Sorghum, BGN flour	Sudanese *kissra* bread	Complimentary meal	Abdualrahman *et al.* (2019)
Sorghum, BGN	*Mahewu*	non-alcoholic beverage	Qaku *et al.* (2020)
BGN, whole wheat	Bread	composite meal	Yusufu & Ejeh (2018)
BGN, palm plant husk ash	*Okpa*	Fortified snack	Akande & Fabiyi (2010)
unripe plantain, BGN, and turmeric	Snack	Snack	Adegunwa *et al.* (2017)

n/a: Not applicable

2.2.2 Malting of Bambara Groundnut

Malting has been used extensively to improve the nutrition and functionality of legumes (Pal *et al.*, 2017; Setia, 2019; Kumar *et al.*, 2020). The difficulty in processing BGN has led researchers to develop different processing methods (Tan *et al.*, 2020). The processing methods employed go a long way to determine the nutritional value of BGN, as is the case for other legumes (Dziki & Gawlik-Dziki, 2019). Malting has been used to improve nutritional components, reduce anti-nutritional and toxic factors that may likely interact with BGN food nutrients (Yao *et al.*, 2015).

Bambara groundnut is known for its 'hard to mill" phenomenon, one of the major factors limiting its use (Khan *et al.*, 2021). However, malting has been used to make the seeds brittle, easily breakable and milled into flour, and boost flour extraction rate (Uvere *et al.*, 1999; Viktorinová *et al.*, 2020; Krapf, 2021). In addition, germination has been used to improve the total phenolic content of BGN and reduce the tannins, trypsin inhibitors, phytate, lectins and flatus oligosaccharides (Okafor *et al.*, 2014; Nyau *et al.*, 2017; Chinma *et al.*, 2021; Khan *et al.*, 2021). The effect of germination on the anti-nutrients are shown in Table 2.6.

Table 2.6 Effect of germination on Bambara groundnut flour anti-nutritional factors and total phenolic

	Germination time (h)			
Anti-nutritional factors and Total Polyphenolic Content	**Raw flour**	**24**	**48**	**72**
Phytic acid (mg/100 g)	4.75	3.48	2.86	2.32
Tannin (mg CE/g)	5.13	4.37	3.51	1.86
Trypsin inhibitory activity (TIU/mg)	2.4	1.82	1.26	0.63
Total phenolic content (µmol TE/g)	1.15	1.58	1.81	2.03

Source: Chinma *et al.* (2021)

The phytic acid contents of BGN decreased after germination for 24 h (3.48 mg/100 g), 48 h (2.86 mg/100 g) and 72 h (2.32 mg/100 g) and the reduction was attributed to the activation of phytase enzyme which degraded phytate to yield nutrients such as inositol and phosphate. Trypsin inhibitor activity was also reduced with increased germination time compared to the raw BGN seeds. The reduction was attributed to the enzymatic degradation of proteins such as the protease inhibitors responsible for the trypsin inhibitors inactivation, which is a proteinaceous antinutritional compound. The increase in the total

phenolic content of germinated BGN flours ranged from 1.58, 1.81, 2.03 µmol TE/g for 24, 48, and 72 h, respectively. The increase was attributed to the activation of the key enzyme in phenolic biosynthesis, phenylalanine ammonia-lyase, which encouraged the formation of phenolic compounds in germinated seeds.

A high loss in raffinose and stachyose content was observed after malting Bambara groundnut commercial (67 and 64%), Bambara groundnut white (76 and 56%) and Bambara groundnut brown (82 and 71%) as shown in Table 2.7 (Adeleke *et al.*, 2017b). Studies have also shown that germination improved the amino acid composition of BGN and increased *in vitro* protein digestibility, as shown in Tables 2.8 and 2.9 (Chinma *et al.*, 2021).

Table 2.7 Loss of raffinose and stachyose in 72 h germinated Bambara groundnut

Samples	**Raffinose**		**Stachyose**	
	Concentration (g/100g)	**Loss (%)**	**Concentration (g/100g)**	**Loss (%)**
Raw seeds	2.05	-	1.48	-
Bambara groundnut commercial (BGC)	0.68	67	0.53	64
Raw seeds	1.30	-	0.86	-
Bambara groundnut white (BGW)	0.31	76	0.35	56
Raw seeds	1.79	-	1.22	-
Bambara groundnut brown (BGW)	0.33	82	0.35	71

Source: Adeleke *et al.* (2017b)

Table 2.8 In vitro protein digestibility reduction in germinated Bambara groundnut

	Sprouting time (h)			
In vitro protein digestibility[1]	**Raw flour**	**24**	**48**	**72**
Protein digestibility IVPG (%)	73.67	83.10	86.44	88.90
IVPD increase (%)		12.8	17.33	20.67

[1]IVPD–*In vitro* protein digestibility

Source: Chinma et al. (2021)

Table 2.9 Essential and non-essential amino acid profile of raw and malted Bambara groundnut

Amino acids (g/100g)	**Germination time (h)**			
Essential amino acid	**Raw flour**	**24**	**48**	**72**
Histidine	0.55	0.63	0.68	0.67
Isoleucine	0.63	0.71	0.82	0.85
Leucine	1.20	1.33	1.41	1.53
Lysine	1.13	1.25	1.37	1.45
Methionine	0.20	0.23	0.31	0.33
Phenylalanine	0.76	0.87	0.96	1.04
Threonine	0.61	0.69	0.75	0.86
Valine	0.74	0.83	0.91	0.98
Total essential amino acid (TEAA)	5.82	6.54	7.21	7.71
TEAA increase (%)	-	12.37	23.88	32.47
Non-essential amino acid				
Alanine	0.67	0.73	0.79	0.85
Arginine	1.25	1.18	1.10	1.02
Aspartic acid	2.20	2.32	2.41	2.68
Cysteine	0.23	0.28	0.33	0.36
Glutamic acid	3.02	3.13	3.24	3.35
Glycine	0.68	0.77	0.83	0.91
Proline	0.45	0.52	0.61	0.69
Serine	0.81	0.89	0.97	1.05
Tyrosine	0.52	0.57	0.61	0.73
Total non-essential amino acid	9.83	10.27	10.89	11.64
TNEAA increase (%)	-	4.48	6.31	18.41

Values on a dry weight basis. TEAA-Total essential amino acid, TNEAA-Total non-essential amino acid [Source: Chinma *et al.* (2021)]

The functional properties of raw and sprouted Niger cream (72 h sprouting time) and Yobe black (92 h sprouting time) BGN landraces were improved (Abba *et al.*, 2018). The protein isolate produced from the sprouted BGN exhibited improved foaming capacity and stability, as shown in Table 2.10. In addition, the sprouting of BGN significantly led to an increase in emulsion properties, water absorption properties, swelling index and bulk densities in the two varieties. The functional characteristics of the sprouted BGN landrace showed improvement in the quality and properties of its protein. Therefore, having a product

with good quality functional properties could provide a cheap protein source that can be introduced in food industries to produce protein-rich products that could combat malnutrition and undernutrition.

Table 2.10 Effect of germination on functional properties of BGN flour

Parameter	Raw Niger cream	Sprouted Niger cream (72 h)	Raw Yobe black	Sprouted Yobe Black (92 h)
Yield (%)	19.60	17.30	22.37	16.70
Swelling index (mL)	0.59	0.52	0.52	0.48
Bulk density (g/mL)	0.67	0.72	0.64	0.17
Emulsion capacity (%)	38.17	39.30	37.61	38.34
Emulsion stability (%)	38.94	43.61	39.45	41.35
Water absorption (%)	6.43	6.66	6.20	6.50
Oil absorption (%)	3.05	3.51	2.97	3.42
Foaming capacity (%)	11.00	35.00	12.67	17.67

Source: Abba et al. (2018)

Investigating the chemical, sensory and rheological properties of porridges from sprouted sorghum, BGN, and fermented sweet potato showed that amylase-rich food products could be produced from malted BGN (Nnam, 2001). The decrease in carbohydrates was attributed to the increased activities of α and β-amylases during the metabolic activities induced by the malting process (soaking and sprouting phases). The activities of α and β-amylases increased as malting progressed with reduced carbohydrates. The α and β-amylases acted on the starch granules to break down the amylose chains by degrading the starch molecules into simple sugars (Lemmens *et al.*, 2019). Amylases are easily activated during the malting process than any other food processing method due to the breakdown of carbohydrate components (Onukogu, 2021). Malted grains are thus pre-digested food ingredients that are important in the food industry (Boye, 2015; Vasantharuba, 2016).

Malting, most especially the soaking and sprouting stage, being a simple biotechnological technique, increases enzyme activities and many bioactive activities that improve and enhance the biological constituents of grains, thereby resulting in pre-digestion of carbohydrate and protein (Vasantharuba, 2016). Thus, the beneficial changes in the nutritional and functional properties of BGN malted. These beneficial effects of malting make BGN suitable for applications in food systems such as weaning foods, bakery

products and beverages (Akpapunam *et al.*, 1996). Subjecting BGN and its malt product to metabolic profiling will encourage potential users to understand the bioactivity during the malting process of BGN and increase its use industrially. However, the metabolite profiling of germinated BGN flour and starch by Oyedeji *et al.* (2021) showed that the metabolite compounds identified could be a better means of understanding its constituents and can lead to utilization of the compounds for functional food production.

2.3 Metabolomics in Food Science

Metabolomics is an important tool in agriculture, pharmacy, and environmental sciences. The metabolome is the collection of all small molecule metabolites or chemicals found in a cell, organ, or organism. These small molecule metabolites or chemicals include endogenous and exogenous chemical entities such as peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids, minerals and just about any other chemical that can be used (Wishart, 2008). Recently there has been an increase in compound extraction, separation, detection, identification, and data analysis. It aims to detect, identify, and quantify a wide range of compounds from different chemical classes to provide a comprehensive image of the metabolic profiles in various biological systems. The application of metabolomics has helped food processing, quality, and safety in food science (Cevallos-Cevallos *et al.*, 2009; Cevallos-Cevallos & Reyes-De-Corcuera, 2012).

In food science, metabolomics, called foodomics, study many small metabolites in a system that has become an important tool in many research areas (Cifuentes, 2013). Foodomics was first defined in 2009 ‘as a discipline that studies the food and nutrition domains through the application and integration of advanced omics technologies to improve consumer's well-being, health, and confidence’ (Mendiola *et al.*, 2013; Cifuentes, 2015, 2017). It is an integrative structure that incorporates transcriptomics, proteomics, and metabolomics. Foodomics covers research areas such as food safety, traceability, quality, and bioactivity (Valdés *et al.*, 2021; Yang *et al.*, 2021). Its application helps improve consumer wellness, health, and confidence in food (Álvarez-Rivera *et al.*, 2021), as illustrated in Figure 2.2. It has risen as a tool for quality, processing, and safety of raw materials and final products (Cevallos-Cevallos *et al.*, 2009; Ibáñez *et al.*, 2012; Schasteen, 2016). The general goal of most metabolic profiling is to generate the metabolic activities of the biological state of a sample and describe the changes happening during a process (Cifuentes, 2012). It also measures the metabolites in relation to natural and controlled conditions like malting (Schuhmacher *et al.*, 2013).

Malting is a controlled process and an important food processing application in food science due to its involvement in lots of bioactivities to improve grains and is beneficial to human health (León *et al.*, 2018; Ikram *et al.*, 2021). The process, involving germination of

grains after initial soaking and kiln drying of the germinated kernels are crucial steps, playing a central role in the final product with a spectrum of significant changes in metabolite contents (Márton *et al.*, 2010; Silva *et al.*, 2020; Álvarez-Rivera *et al.*, 2021). This makes malting an ideal system to evaluate metabolic variation because of the metabolic changes during the malting process (Aguilera *et al.*, 2013; Singh *et al.*, 2015; Baranwal, 2017; Hingade *et al.*, 2019). First, the dormant enzymes and proteins are activated, then complex oligosaccharides break down into simple fermentable sugars (Davies, 2016; MacLeod & Evans, 2016). Malts, therefore, represent processed seed tissue whose metabolites contribute directly to the energy required for fermentation, flavour, aroma, and appearance characteristics (Rosentrater & Evers, 2018; Cash, 2021). The use of non-targeted liquid chromatography-mass spectrometry metabolite profiling on barley grain and malt showed that malted barley was more influenced by genotype compared with un-malted barley grains (Heuberger *et al.*, 2014). Metabolomics or metabolic profiling process has proven to be an effective tool for analysing and investigating germinated grains, plants and animals (Frank *et al.*, 2011; Jom, 2012; Oyedeji *et al.*, 2021).

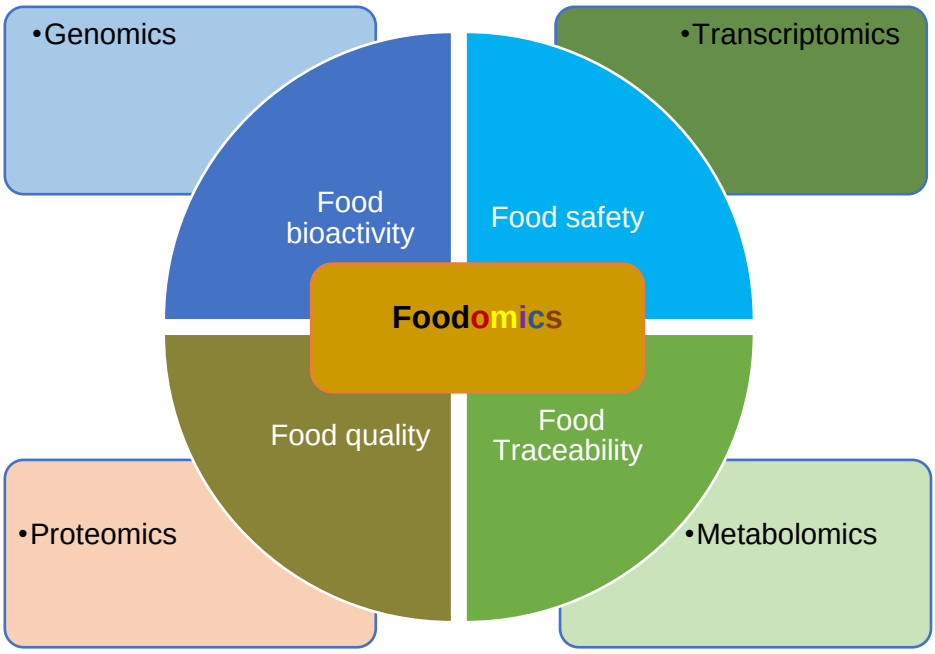

Figure 2.2: Recent tools and applications of Foodomics

Some of the recent metabolomics analyses in food science, as compiled by Cevallos-Cevallos & Reyes-De-Corcuera (2012), are listed in Table 2.11. The metabolism

Table 2.11 Some recent metabolomics analysis in food science

Sample	Study	Separation–detection	Data analysis	References
Chicken feed	Food quality/untargeted	NMR, LC–MS	PCA, ANOVA	Ruiz-Aracama, Lommen, Huber, Va De Vijver, & Hoogenboom (2012)
Milk	Food quality/untargeted	IR	PLS	Aernouts, Polshin, Saeys, ar Lammertyn (2011)
Rice	Food quality/untargeted	GC–MS, LC–MS, CE–MS	PLS, ANOVA	Redestig et al. (2011)
Tomato	Food quality/untargeted	GC–MS	PCA	Luengwilai, Saltveit, and Beckl (2012)
Beer	Food quality/untargeted	TOFMS	PLS	Cajka et al. (2011)
Wine	Food quality/untargeted	GC–FID	PCA, PLS, ANOVA	Malherbe et al. (2012)
Coconut oil	Food quality/targeted	GC–FID	ANOVA	Kumar (2011)
Salmon	Food quality/targeted	NMR	Univariate	Bankefors et al. (2011)
Meat	Food quality/targeted	LC–MS	Univariate	D'Alessandro et al. (2011)
Honey	Food quality/targeted	GC–MS	ANOVA	Bianchi et al. (2011)
Ketchup	Food quality/targeted	LC-MS	ANOVA	Vallverdu-Queralt et al. (2011)
Soy sauce	Food processing/targeted	GC–MS	Univariate	Feng, Zhan, Wang, Zhang, & L (2012)
Soybean, maize	Food toxicology/targeted	HPLC–MS	Univariate	She et al. (2012)

Source: Cevallos-Cevallos & Reyes-De-Corcuera (2012)

of plants and animals are dependent on the components of the food (Almstetter *et al.*, 2012). Food components interact due to external factors before, during, and after processing (Cifuentes, 2012; Laghi *et al.*, 2014; Ellis *et al.*, 2016). The physical and chemical interactions of all compounds present in various food components bring about the product's attributes, including sensory acceptability, quality, safety, and shelf life (Martinović *et al.*, 2016; Jagadeesan *et al.*, 2019; Garwolińska *et al.*, 2021). The need to identify the food components and their interactions under controlled and uncontrolled conditions will give better control and understanding of food processes and systems (Cifuentes, 2013; Braconi *et al.*, 2018). Thus, the use of metabolomics (foodomics) to study the chemical constituents of a system will offer a systematic and comprehensive approach to determine, quantify, and identify food components that will influence the characteristics of a final product and its effect on human health (Cevallos-Cevallos & Reyes-De-Corcuera, 2012; Chaudhary *et al.*, 2021).

2.3.1 Metabolomic Profiling of Bambara Groundnut and Its Products

Recently, BGN has also been profiled by some researchers for their potential nutritional and anti-nutritional characteristics. The identification of the nutraceutical phenolic compounds in BGN was performed by HPLC-PDA-ESI-MS, where a total of 27 phenolic compounds were identified in the red and brown BGN varieties (Nyau *et al.*, 2015). The phenolic compounds identified were mainly phenolic acids and flavonoids consisting of quinic acid, (E) GC-hexoside, catechin glucoside, catechin, epicatechin, medioresinol, p-coumaric acid, salicylic acid, caffeic acid derivative, catechin dimer, myricetin hexoside, quercetin-3-O-rutinoside and quercetin-3-O-glucoside. The study showed that BGN had beneficial phytochemical constituents that have protective functions. Also, untargeted metabolites analysis of the phytochemical profile of seeds from 21 BGN landraces via UPLC-q TOF-MS (Ultra performance liquid chromatography- Quadrupole time of flight mass spectrometry) by Tsamo *et al.* (2018). Metabolites identified were flavonoid conjugates consisting of catechin, quercetin, kaempferol, apigenin, and other metabolites such phenolic acid, saponins, sphingolipids, and fatty acids. The study indicated that BGN metabolites have good nutraceutical properties that could benefit the healthy living, but the metabolic activities of malted BGN showed improved metabolites. The use of High-Performance Liquid Chromatography-Photo Diode Array-Electrospray Ionization-Mass Spectrometry (HPLC-PDA-ESI-MS) profiling of phenolic phytochemicals in sprouted common beans and BGN methanolic extract sprouted for 8 days showed that sprouting had a positive effect on the total polyphenol concentration of the BGN methanolic extract with an increase of up to 1.3-fold compared to the unsprouted seeds (Nyau *et al.*, 2017).

Profiling the metabolite of BGN and *dawadawa* produced from un-hulled and dehulled BGN seeds using Gas chromatography high-resolution time-of-flight mass spectrometry (GC-HRTOF-MS), Adebiyi *et al.* (2021) detected esters, ketones, phenols and many flavour compounds. The compounds identified contributed to the sensory properties, nutritional and functional properties of BGN and the products. The study concluded that the beneficial components could be incorporated into human diets for their health benefits.

The metabolite profiling of germinated BGN flour and starch was carried out by Oyedeji *et al.* (2021). The germinated samples were extracted using methanol/water (80:20v/v) and acetonitrile/methanol/water (40:40:20 v/v/v) and analysed using the gas chromatography-high resolution time of flight mass spectrometer (GC-HRTOF-MS). The metabolites identified were grouped into acids, alcohols, cyclic compounds, esters, ketones, phytosterols and vitamins. The study reported the effect of different extraction methods on the germinated BGN flour and starch metabolite compared to the ungerminated.

2.3.2 Process of Metabolites Profiling

Metabolic profiling is classified as targeted (specific) or untargeted (non-selective) analyses. The targeted analysis is used for particular metabolites that require identifying and quantifying. The importance of these types of metabolites is to evaluate the behaviour of a specific group of compounds in a sample under specified conditions. On the other hand, untargeted metabolic profiling is used to detect many metabolites; it doesn't quantify any specific compound (Mozzi *et al.*, 2013). The key steps in metabolic profiling are separating and detecting the metabolite, as illustrated in Figure 2.3. Some of the techniques used in separation are liquid chromatography (LC) in its high performance (HPLC) or ultra-performance (UPLC) forms, gas chromatography (GC), capillary electrophoresis (CE). Then, they are detected with techniques such as mass spectrometry (MS), nuclear magnetic resonance (NMR), flame ionization detector (FID) and near-infrared spectrometry (NIR) (Roessner & Bowne, 2009; Kahl, 2010; Wood, 2021). In food metabolomics, mass spectrometry (MS) and nuclear magnetic resonance (NMR) have been used the most (Wishart, 2008; Cevallos-Cevallos *et al.*, 2009; Oms-Oliu *et al.*, 2013; Cubero-Leon *et al.*, 2014; Kim *et al.*, 2016; Muhialdin *et al.*, 2020).

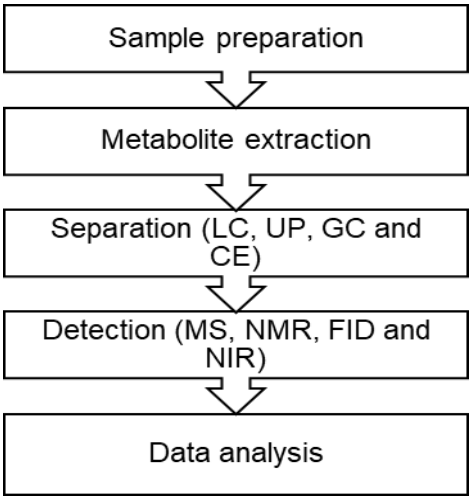

Figure 2.3: Compound identification in metabolomics analyses

2.4 Conclusions

The nutritional, functional, organoleptic, colour and textural properties of malted grains make them very desirable in the food industry compared to non-germinated grains. The balanced nutritional content of BGN, consisting of carbohydrate, protein, and fat, makes it a potential food crop that can be malted and used as a functional ingredient or condiments in the household, food, and beverage industries. Malting the BGN seeds will allow access to its bioactive components that can be beneficial to consumers. Thus, producing enzyme-rich food ingredients and condiments from malted BGN seeds. It can also produce biologically active compounds, which have potential applications in functional food, nutraceutical, pharmaceutical, and cosmetic market sectors. Furthermore, successful production of malted BGN seeds, analysis of their metabolites and physicochemical characteristics will also promote the utilisation of BGN and encourage its production with added income to the farmers.

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CHAPTER THREE

ENZYME AND ANTIOXIDANT ACTIVITIES OF MALTED BAMBARA GROUNDNUT AS AFFECTED BY STEEPING AND SPROUTING TIMES

Abstract

Bambara groundnut [BGN] is termed a complete food due to its nutritional composition, and this has made it to be researched to have access to its nutritional constituents. It has gone through various processing methods, including soaking, cooking, dehulling and malting. Malting BGN seeds have shown that the nutritional components improved, and anti-nutritional reduced; however, there are no or little studies on the enzyme and antioxidant activities of malted BGN as affected by steeping and sprouting times. Bambara groundnut was malted by steeping in distilled water at 25 ± 3^{o}C for 36 and 48 h and then sprouted for 144 h at 30^{o}C and samples were drawn every 24 h for drying to study the steeping and sprouting times effect on the physicochemical properties, enzymes, and antioxidant activities of the BGN seeds. The moisture, sprout length, pH, colour, protein content, amylase, total polyphenols, and antioxidants activities were determined. The sprout length was measured using the vernier caliper, pH by the laboratory pH meter. The colour was measured using the colour Flex EZ 25 mm aperture set for daylight illumination D65 and a 10^{o} standard observer angle. The α- and β-amylase activities of the sprouted samples were analysed by the enzymatic Ceralpha Method (K-CERA, Megazyme) kit and enzymatic Beta-Amylase (Megazyme, K-BETA3) kit, respectively. The Folin–Ciocâlteu reagent (FCR), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay methods were used to determine the polyphenolic and antioxidants activities. The moisture and sprout length increased with increased steeping and sprouting times; however, the 48 h steeped seed sprouts were lower than the 36 h steeped seeds. The steeping and sprouting times significantly affected the BGN malt colour quality and pH. There was no significant difference in the protein content of the BGN seeds based on steeping and sprouting times. Steeping and sprouting times significantly affected the α- and β-amylase activities of the BGN seeds. The activity of amylases differed significantly for the 36 and 48 h steeping time, with mean α-amylase of 0.16 and 0.15 CU/g, respectively, β-amylase of 0.22 and 0.23 BU/g for the two steeping times. The optimal condition for malting BGN seeds for an α-amylase-rich (0.18 CU/g) malt was 36 h steeping and 96 h germination while for β-amylase-rich (0.30 BU/g) malt steeping at 48 h and sprouting for 72 h. The total polyphenolic content increased significantly with increased steeping and sprouting times. There was a significant ($p = 0.000$) difference between FRAP and DPPH antioxidant

concentration in the 36 and 48 steeping times; however, sprouting from 24 to 144 h did not show a significant difference.

3.1 Introduction

Malting is the incomplete germination of cereal grains and pulses under controlled water, temperature, and humidity (Briggs, 1998; Lewis et al., 2001; Mulder, 2005; MacLeod & Evans, 2016; Baranwal, 2017). It involves three stages: steeping, sprouting, and drying (kilning), carefully monitored because every process stage affects the end product (Edney & Izydorczyk, 2003; MacLeod & Evans, 2016). The main objective of malting is to encourage hydrolytic enzyme development because un-germinated cereals and legumes cannot develop enzymes (Baranwal, 2017; Rosentrater & Evers, 2018). Sprouting and acrospires form during the malting process, and enzymes become active, altering the grain structure and resulting in a malt product; used in the brewing, food, and beverage industries. The sequence of substantial changes in metabolite and enzyme activity in the resultant malts, on the other hand, is time-dependent (Frank et al., 2011a; Arendt & Zannini, 2013; MacLeod & Evans, 2016). The seed embryo releases gibberellic acid (GA), which moves to the aleurone to induce hydrolytic enzymes (Møller & Svensson, 2021). The enzymes released catalyse the breakdown of cell wall reserves (polysaccharides and starchy energy reserves) necessary for sprouting and malt production (Aubert et al., 2018; Guzmán-Ortiz et al., 2019; Møller & Svensson, 2021).

The malting process metabolically makes the protein soluble, increases the enzyme activity, breaks down starch into simple sugars, and develops colour and flavour (Gupta et al., 2010; MacLeod & Evans, 2016; Møller & Svensson, 2021). The essential enzymes for the diastatic power measurements are the α- and β-amylases (MacLeod & Evans, 2016; Lekjing & Venkatachalam, 2020). Apart from the α- and β-amylases that hydrolyse starch, there are many metabolic changes and modifications during sprouting and malting (Briggs, 1998; Joshi, 2018; Møller & Svensson, 2021). Sprouting seeds in plant science signifies a vital stage depending on time (Shu *et al.*, 2008; Frank *et al.*, 2011; Xue *et al.*, 2016). The steeping, sprouting, and drying times affect the changes and modifications in the malted cereals and legumes (Lewis & Young, 2001; Woffenden et al., 2002a,b; Gorzolka et al., 2012; Gebremariam et al., 2013; López-Cortez et al., 2016; Hung et al., 2020a).

Sprouting is an effective and inexpensive technology to improve (modification and increase nutritional components) cereals and legumes grain quality (Chavan *et al.*, 1989; Gharachorloo *et al.*, 2013; Ohanenye *et al.*, 2020; Cash, 2021). Sprouting has been established to improve the nutritional properties, increase the essential nutrients, lower the anti-nutrients and increase the antioxidant content (Chavan et al., 1989; Mwikya et al., 2001; Kuo et al., 2004; Márton et al., 2010; Xu et al., 2019; EL-Suhaibani et al., 2020; Hung

et al., 2020b, a; Ohanenye et al., 2020). There are many physical, chemical, and biological changes inside the seed during the sprouting stage (Cash, 2021). For example, the activation of the hydrolytic enzymes, hydrolysing proteins, polysaccharides, and fats, leads to increased nutritional and antioxidant contents (Medhe *et al.*, 2019; Kumar *et al.*, 2021).

The biological changes in sprouted grains lead to ease of processing (Swanston & Taylor, 1990; Uvere et al., 1999; Xu et al., 2020); creation of colour, odour, flavour, and functional properties (Chavan *et al.*, 1981; Mbithi *et al.*, 2001; Gharachorloo *et al.*, 2013; Xu *et al.*, 2019; EL-Suhaibani *et al.*, 2020; Hung *et al.*, 2020b; Ma *et al.*, 2020; Ohanenye *et al.*, 2020; Winarsi *et al.*, 2020; Ispiryan *et al.*, 2021; Kumar *et al.*, 2021). In addition, it has been established through studies that eating germinated cereals and legumes may help in the reduction of chronic diseases such as cancer, diabetes, hypertension, hyperlipidaemia, obesity, and heart diseases (Márton *et al.*, 2010; Gharachorloo *et al.*, 2013; Ohanenye *et al.*, 2020; Kumar *et al.*, 2021).

Native to north-east China (Manchuria), soybean (*Glycine max*) malting characteristics has been researched, especially on soaking, sprouting, and drying durations (Singh, 2010; Kayembe & Jansen van Rensburg, 2013; Ghani et al., 2016; Hart, 2017). Soybean's malting process improved its nutritional content and removed anti-nutrients (Kaczmarska et al., 2018). In addition, some functional, active components present in low content in un-germinated soybean were increased by germination (Warle *et al.*, 2015; Kaczmarska *et al.*, 2018). Such components include soy isoflavones, γ-aminobutyric acid (GABA), polyphenols, and antioxidants (Zieliński, 2003; Huang et al., 2014; Xu and Hu, 2014; Mamilla & Mishra, 2017; Miglani & Sharma, 2018; Chua et al., 2019; Gan et al., 2019; Wu & Xu, 2019). These components change the nutritional, physical, functional, and health benefits of malted soybeans, which has contributed to the development of healthy soybean food products (Jiang et al., 2013; Zhou et al., 2013; Huang et al., 2014; Murugkar, 2014; Dziki & Gawlik-Dziki, 2019).

Bambara groundnut (BGN), a legume indigenous to Africa, has also been researched to gain popularity like soybean. Bambara groundnut is known for its various nutritional and therapeutic values (Atiku et al., 2004; Murevanhema & Jideani, 2013; Diedericks & Jideani, 2015; Jideani, 2016). Research has proven BGN to be resilient with ability to withstand drought compared to other legumes and able to produce high yields (Heller *et al.*, 1997; Cleasby *et al.*, 2016; Mabhaudhi *et al.*, 2018; Feldman *et al.*, 2019; Mayes *et al.*, 2019; Muhammad *et al.*, 2020). New and improved products have been developed from malted and un-malted (raw BGN) to encourage its use. Some of the new products from BGN through research include yoghurt, milk (powdered and liquid), and value-added snacks (Alobo, 1999; Falade & Nwajei, 2015; Hardy & Jideani, 2016; Pahane et al., 2017).

Bambara groundnut has also been malted to study its chemical properties and functional use in new food formulation and its therapeutic potentials. BGN was malted to investigate the effects on the milling performance and acceptability of the malted seeds for the production of *okpa,* a steamed Bambara groundnut cake(Uvere *et al.*, 1999). Akpapunam et al. (1996) investigated the malting time effect on BGN flour chemical composition and its functional properties. The profiling of the phenolic compounds in sprouted BGN by Nyau *et al.* (2017) showed an increase by 1.3-fold total polyphenol content after sprouting, revealing new emerging compounds. Also, sprouted BGN flours caused a decline in phytic acid, tannin, and trypsin inhibitor, which resulted in the improvement of protein content, insoluble dietary with the enhancement of the trace minerals, amino acids, digestible starch, and in vitro protein digestibility (Chinma *et al.*, 2021). Sprouting has been shown to be effective in the decrease of anti-nutritional components, improving the nutritional and functional properties of BGN (Acquah *et al.*, 2021; Oyedeji *et al.*, 2021). However, there is a need to know the characteristic physicochemical changes that occur during the malting process of BGN seeds. Therefore, this chapter's objective was to establish the physicochemical characteristics, enzymes, total polyphenolics, and antioxidant activities of malted BGN seeds as affected by steeping and sprouting times.

3.2 Materials and Methods

3.2.1 Source of materials, reagents, and equipment

The BGN seeds were purchased from Triotrade Johannesburg, South Africa, and used as received without sorting into the colours. Chemicals and reagents were of analytical standards. Alpha and beta-amylase kits were from Megazyme Ltd, Ireland. All other equipment was from the Department of Food Science and Technology and Oxidative Stress Research Centre, Cape Peninsula University of Technology, Cape Town, South Africa.

The equipment and instruments used in this study were the ten trays hot air Excalibur Food Dehydrator (Excalibur, Sacramento, CA, USA), LECO CN 628 Dumas nitrogen analyser (Leco Corp., St Joseph, MI, USA), the centrifuge (Avanti® J-E centrifuge JSE111330, Beckman coulter Inc., USA), and Thermos Scientific Multiskan plate reader spectrophotometer (Thermo Scientific, USA). Others are the pH meter (Hannah checker pH meter, Model HI1270), a water bath, Colour Flex EZ (Model TC-P III-A, Tokyo Denshoku Co., Ltd., Japan). Figure 3.1 outlines the sample treatments and analyses carried out in this chapter, consisting of the sprout length, moisture, colour, pH, protein content, α and β amylase activities (Ceralpha & Beta-amylase enzymatic kit), Total polyphenols (Folin–Ciocâlteu Reagent Assay (FCR) method) and antioxidant activities (Ferric Reducing

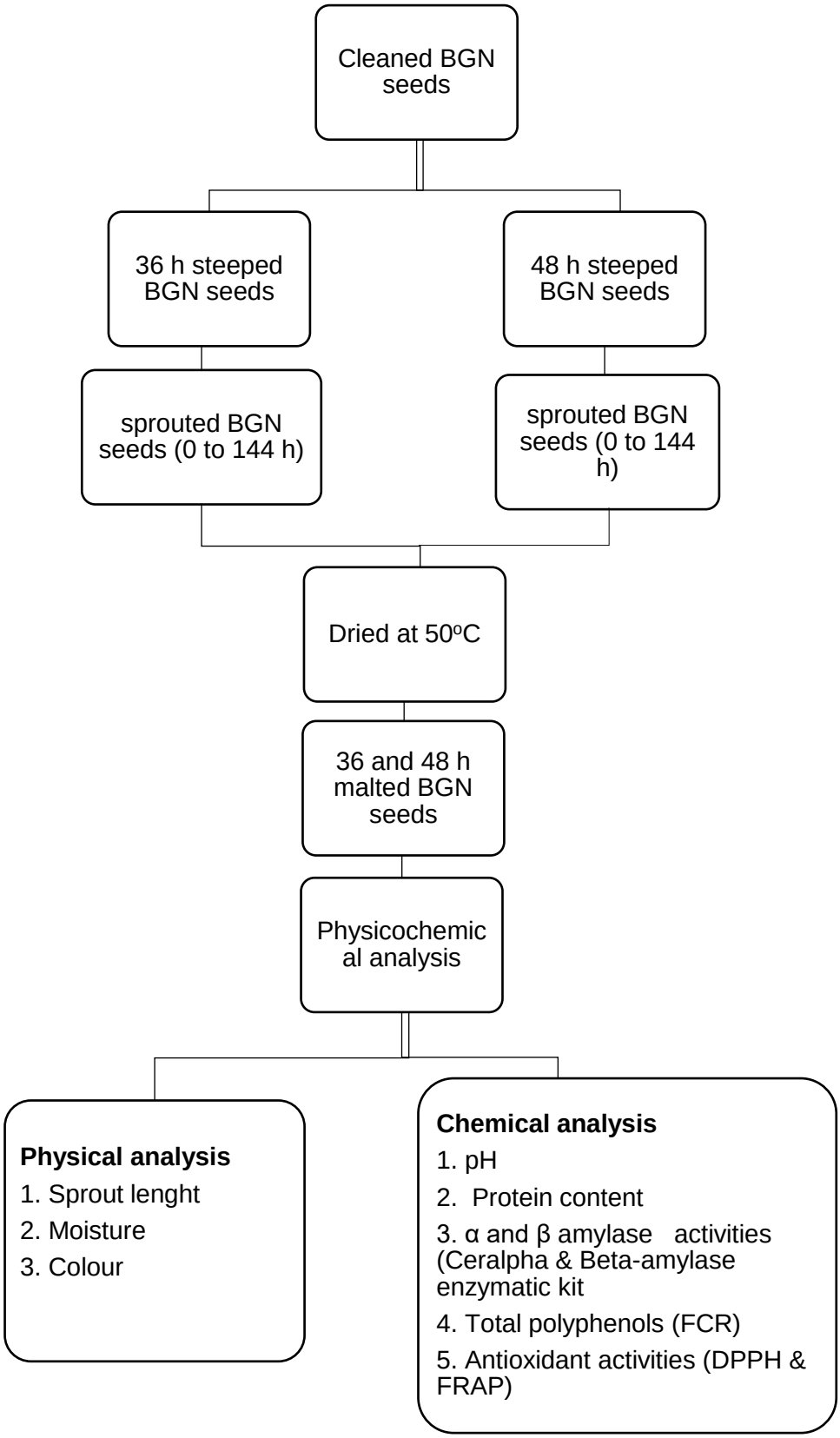


Figure 3.1: Chapter 3 experimental outline

Antioxidant Power Assay (FRAP) and 2,2-diphenyl-1-picrylhydrazyl assay (DPPH) methods).

3.2.2 Malted Bambara groundnut production process

The raw BGN were spread out in trays to facilitate the removal of foreign materials, broken and poorly developed seeds. Distilled water was used to wash the grains to adequately remove dirt and dust particles. The cleaned BGN seeds were divided into two equal portions of 1400 g each and were steeped at 36 and 48 h due to their hard nature (Obizoba & Egbuna, 1992; Jom et al., 2011). Figure 3.2 below outlines the process flow for the BGN malting process.

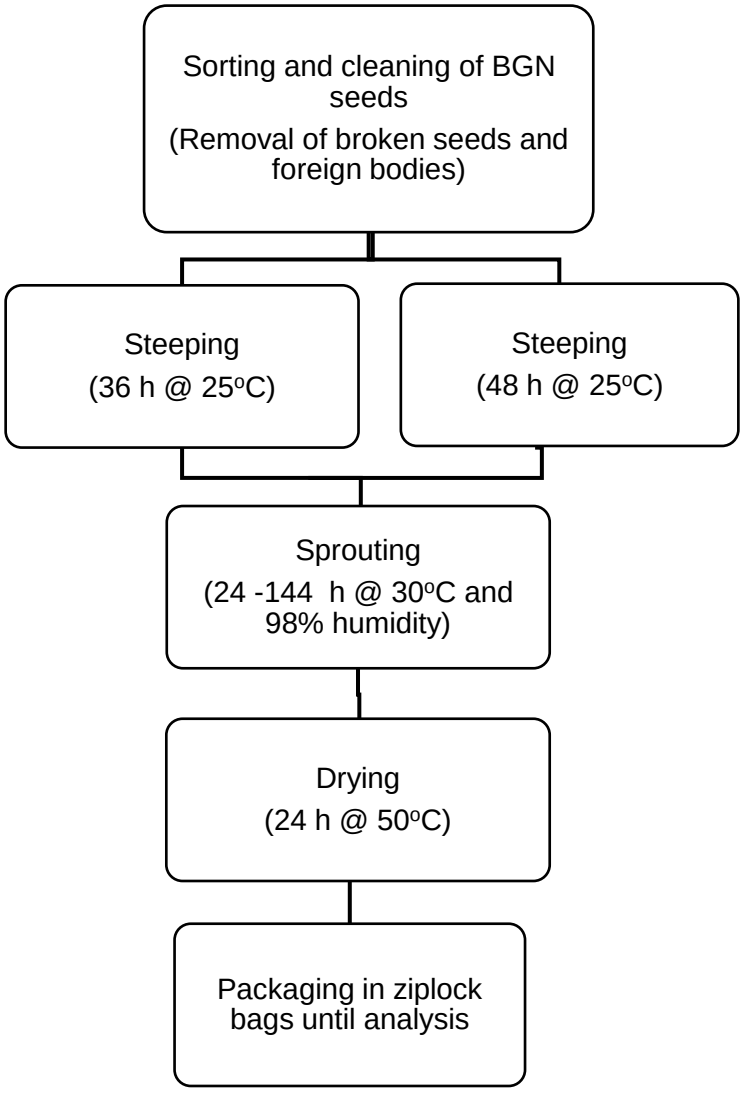


Figure 3.2: Malting process of Bambara groundnut malts

The two batches of cleaned BGN seeds (1400 g) were steeped at 25^{o}C for 12 h, followed by a 12 h air rest (25°C) and a second steep (12 h, 25°C) for 36 h steeped seeds. While the 48 h steeped BGN seeds were steeped at 25°C for 24 h, followed by a 12 h air-rest (25°C) and a second steep (12 h, 25°C). The steeped grains were air-rested 12 hourly by spreading them on 45 by 30 cm plastic tray baskets at 25 ± 3^{o}C to allow air into the grains. Steeping was done with 7 L of distilled water using two 25 L round white buckets for 38 and 48 h at 25^{o}C until they absorbed between 41 to 43% of their initial weight in water to initiate sprouting. The 36 and 48 h steeped BGN seeds increased in weight to 2394 and 2702 g, respectively and were divided into six equal portions. The seeds were spread out on the plastic tray baskets, arranged inside the side-by-side Macadam proofer (2250 by 1000 mm in size) at 99% humidity and 30^{o}C from 24 to 144 h.

Distilled water (10 mL) was sprinkled on the sprouting seeds every 12 h. Ten samples of the sprout length in triplicate from the sprouted seeds for each batch every 24 h were measured using the Vernier calliper. Samples were drawn at 24, 48, 72, 96, 120, and 144 h of germination time and dried in a hot air Excalibur Food Dehydrator (Excalibur, Sacramento, CA, USA) maintained at 55^{o}C for 24 h. The dried samples were milled using Waring Laboratory Science blender model 7009G (Waring Laboratory Science, CT, USA). Then packaged in zip lock bags and stored at −18^{O}C until further analysis. The samples were analysed for the physicochemical characteristics (pH, colour, sprout length, and moisture), amylase, total polyphenolic content, and antioxidant activities.

3.3 Physicochemical Analysis of Bambara groundnut malt

3.3.1 Sprout length and moisture uptake of the Bambara groundnut green malts

The length of the BGN green malt sprouts was measured with the Vernier calliper by measuring ten seeds in triplicate from each day of sprouting following the method of Usansa et al. (2009). The results in cm were the average values from a triplicate set of ten seeds of the malted BGN seeds. According to Alsalman & Ramaswamy (2020), the moisture uptake percentage was determined by measuring the 36 and 48 h BGN seeds on the electronic laboratory balance before steeping. After steeping, the seeds were strained and blotted with a towel to remove the excess water on the surface before weighing. Finally, the moisture uptake percentage (wet basis) was calculated according to equation 3.1.

$$\frac{W_2 - W_1}{W_1} \times 100 \qquad \text{Equation 3.1}$$

W1 is the weight before steeping, and W2 is after steeping.

3.3.2 pH determination of Bambara groundnut malts

Following the method of Atudorei et al. (2021), a slurry with 10 g milled BGN malt and 40 mL distilled water was prepared in 50 mL centrifuge tubes. The vortex mixer was used to mix the BGN malt and distilled water thoroughly. The mixtures were kept at room temperature for 1 h and centrifuged at 1500×g for 10 min. The decanted liquid pH was measured in triplicate using a laboratory pH meter (Hannah checker pH meter, Model HI1270), standardised with buffers 4 and 7.

3.3.3 Colour determination of Bambara groundnut malts

The samples' colour measurements were analysed using Colour Flex EZ (Hunter Lab, Reston, VA, USA), 25 mm aperture set for daylight illumination D65, and 10° standard observer angle following the method of Sofi et al. (2020). The instrument's calibration was done using standard black (L* = 8.47, a* = -0.96, b* = 2.79) and white (L*93.41, a*-1.18, b*0.75) tiles.

The colour coordinates measurement was in triplicates by measuring 5 g of the milled malt samples into a glass sample cup (Hunter Lab 04720900, 6.4 cm) with an internal diameter of 6.4 cm following the method by Panghal et al. (2019). Measurement was by using the Commission Internationale de l'Eclairage's (CIE) L*a*b*, where L* (0 = black and 100 = white), a* (− a* = greenness, and + a* = redness) and b* (− b* = blueness and + b* = yellowness). The chroma and hue values were calculated using the method of Medhe et al. (2019), as shown in Equations 3.1 and 3.2.

$$C = \sqrt{a^{*2} + b^{*2}} \qquad \text{Equation 3.2}$$

Where C = Chroma; a* = redness; b* = yellowness

$$\mathbf{h^{o} = \tan^{-1}\left(\frac{b^{*}}{a^{*}}\right)} \qquad \text{Equation 3.3}$$

Where h^{o}= Hue angle; a* = redness; b* = yellowness

3.3.4 Bambara Groundnut Malts Protein Content Determination

The Association of Official Analytical Chemists (AOAC, 2000) Dumas Nitrogen analysis recommended method was adopted to determine nitrogen content using the LECO CN 628 Dumas nitrogen analyser (Leco Corp., St Joseph, MI, USA). Five blanks, EDTA standard, and ProNutro control sample were first analysed and then samples in duplicate to the value of 0.09 mg, wrapped and tightly folded in tin foil cups P/N: 502-186-200.

The samples combustion was carried out in pure oxygen at a temperature of 950^{o}C in the reactor consisting of the combustion catalyst where a gaseous mix containing carbon dioxide, water, and nitrogen is formed (CO_2, H_2O, NO, NO_2). The designated columns then absorbed the gases, removed oxygen, and converted nitrogen oxides into nitrogen. The residual CO_2 and H_2O were extracted by passing through a thermal conductivity column carried by helium gas. The Dumas Nitrogen analyser measured the nitrogen content. The crude protein was calculated by multiplying the measured nitrogen by the protein factor of 6.25 expressed in percentage following Awobusuyi & Siwela (2019) and Moore et al. (2010).

3.4 Determination of α- and β-amylase Activities of BGN Malts

Montanuci et al. (2017) method of α- and β-amylases activity determination during the malting process was followed. The α- and β-amylase enzymes were determined through the enzymatic Ceralpha kit (K-CERA, Megazyme) and the enzymatic kit Beta-amylase (Megazyme, K-BETA3), respectively as detailed in sections 3.4.1 and 3.4.2. All analyses for enzymatic activity were done in triplicate.

3.4.1 Alpha-amylase assay procedure (Ceralpha method)

The milled 36 and 48 h steeped BGN malts of 3 g were measured separately into 50 mL conical flasks. To each flask, 20 mL of extraction buffer solution of pH 5.4 was added, and the contents of the flask were stirred vigorously using the vortex mixer. The samples were then allowed to extract for 20 min at 40^{o}C in the incubator and occasionally stirred with a vortex mixer. After extraction, 25 mL of each sample were measured into 50 mL centrifuge tubes and centrifuged using the Centrifuge 5810R at 1,000 g for 10 min. Finally, the sample extracts were decanted into 25 mL centrifuge tubes for the assay procedure.

The assay was carried out by measuring 0.2 mL aliquots of Megazyme un-buffered amylase HR reagent into 25 mL centrifuge test tubes. It contains blocked p-nitrophenyl maltoheptaoside (BPNPG7, 54.5 mg) and thermostable α-glucosidase (125 U at pH 6.0). The two were pre-incubated at 40^{o}C for 5 min. The 0.2 mL sample extracts were also pre-incubated at 40^{O}C for 5 min and added directly to the tubes' containing the 0.2 mL of the amylase HR reagent solution. These were incubated at 40^{o}C for 20 min, and precisely 3.0 mL of stopping reagent containing 10 g of tri-sodium phosphate in 1 L of distilled water pH adjusted to 11.0 was added. The contents of the tube were vigorously stirred using the vortex mixer. The absorbance of the solutions was read in triplicate using the Thermo Electron Corporation Multiskan Spectrum set at 400 nm against distilled water.

3.4.2 Beta-amylase assay procedure (Betamyl-3 method)

The milled 36 and 48 h steeped BGN malts of 0.5 g were weighed into 25 mL centrifuge tubes. Five mL extraction buffer (Tris/HCl 25 mL, 1 M, pH 8.0 plus disodium EDTA of 20 mM and sodium azide of 0.02% w/v diluted in distilled water) was added into the sample tubes. The enzymes were allowed to extract for 1 h at room temperature, with repeated stirring on the vortex mixer. The mixtures were centrifuged using the Eppendorf Centrifuge 5810/5810 R at 2,000 g for 10 min. Immediately after centrifugation, 0.2 mL of the filtrate were added to 4.0 mL of the dilution buffer containing MES dilution buffer 48 mL, 1 M, pH 6.2 plus disodium EDTA 20 mM, BSA 10 mg/mL, and sodium azide of 0.09% w/v. This mixture was then used for the assay of β-amylase activities.

The assay of the β-amylase was done by dispensing an aliquot of 0.2 mL of the diluted BGN malt samples into the 25 mL centrifuge tubes. The tubes were pre-incubated at 40^{o}C for 5 min. After incubation, 0.2 mL of pre-incubated Megazyme Betamyl-3 substrate solution containing p-nitrophenyl-β-D-maltotrioside (PNPβ-G3) plus β-glucosidase (50 U) and stabilisers were added to each diluted sample and stirred on the vortex mixer. These mixtures were incubated at 40^{o}C for 10 min. After that, 3.0 mL of the stopping reagent (10 g of Tris buffer (Megazyme cat. no. B-TRIS500) in 900 mL of distilled water, pH adjusted to 8.5) was added. The contents were mixed using a vortex mixer. The absorbance of the solutions was read at 400 nm against distilled water using a Thermo Scientific Multiskan microplate spectrophotometer.

3.5 Determination of Total Polyphenols and Antioxidants Activities of Bambara Groundnut Malts

The Folin–Ciocâlteu reagent (FCR), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay methods used for the determination of polyphenolic and antioxidants activities were followed (Xu & Chang, 2008a; Nemzer et al., 2019; Rico et al., 2020) as detailed in sections 3.61 to 3.36.

3.5.1 Total polyphenols activities determination by Folin–Ciocâlteu Reagent Assay (FCR) method

The analysis used the Folin-Ciocâlteu reagent with Gallic acid as the standard to quantify total polyphenols in BGN malts. The phenolic contents were determined by weighing 500 mg of each sample into screw-cap tubes. The BGN malt samples extraction was carried out with 10 mL of 70% methanol mixed with 0.1% HCL using a vortex mixer. The mixtures were then centrifuged using the Eppendorf Centrifuge 5810/5810 R at 4000 g, 21^{o}C for 5 min. The supernatant was analysed using the Folin–Ciocâlteu assay. Twenty-five microliters of the sample's supernatant were mixed with 125 µl of 0.2 M Folin–Ciocâlteu

reagent and 100 µl of 7.5% Na_2CO_3 solution in a 96-well clear plate. The absorbance was read with a Thermos Scientific Multiskan microplate spectrophotometer reader (734 nm at 25^{o}C) after a 2 h incubation period. The standard calibration curve was constructed with 40 mg gallic acid (Sigma Cat Nr: G7384). The results were expressed as mg Gallic acid equivalents (GAE/g).

3.5.2 Antioxidant activities determination by Ferric Reducing Antioxidant Power Assay (FRAP) method

Five hundred milligrams (500 mg) of the 36 and 48 h steeped BGN malts were weighed into 50 mL screw-cap tubes. Ten millilitres of 70% methanol (containing 0.1% HCl) were added to the samples of the screw-cap tubes. The samples were mixed with a vortex then centrifuged at 4000 rpm for 5 minutes. The supernatants (10 µl each) were pipetted into microplate wells in triplicates. Three hundred microliters (300 µl) of the FRAP reagent were added to each sample in the microplate wells. The samples were incubated for 30 min at 37^{o}C, and absorbance was read at 593 nm using the Thermos Scientific Multiskan microplate spectrophotometer. The results were expressed as mg ascorbic acid equivalents (AAE/g).

3.5.3 Antioxidant activities determination by 2,2-diphenyl-1-picrylhydrazyl assay (DPPH) method

The 36 and 48 h steeped BGN malts free radical scavenging ability was determined using the DPPH radical (25 mg/l) in 70% methanol. Each of the samples was mixed with 0.275 mL DPPH solutions. The samples and standards were incubated at 37^{o}C for 30 min in the dark, and absorbance was read at 517 nm using the Thermos Scientific Multiskan microplate spectrophotometer. The standard was Trolox, and results were expressed as µmole Trolox/g.

3.6 Statistical Data Analysis

IBM Statistical Package (IBM SPSS, version 26, 2018) was used for data analysis. All data were collected in triplicate, and results were expressed as the mean ± standard deviation. The results were subjected to multivariate analysis of variance (MANOVA) when normality is not violated and the Kruskal-Wallis H test when normality is violated to determine the mean differences between treatments. Duncan's multiple range tests were conducted to separate the means where differences existed at $p \leq 0.05$ (IBM SPSS ver 26).

3.7 Results and Discussion

3.7.1 Water absorption of steeped Bambara groundnut seeds

There was a 41.5% increase in water uptake after the BGN seeds' steeping for 36 h and a 48.2% increase for the 48 h steeping. The increase showed a difference of 6.66% in water uptake of the two steeping regimes indicating that the steeping time affected the water uptake of the seeds, as stated by Usansa et al. (2009) and Onwurafor et al. (2020). Legume seeds such as BGN have hard seed coats that make them impermeable to water (Mubaiwa *et al.*, 2017). The slow water uptake of legumes has been attributed to their hilum (Mandizvo & Odindo, 2019). The hilum is a scar on the legume seeds that marks the attachment point to the seed, where water enters the seed coat and cotyledon (Swanson *et al.*, 1985). Once the seed coat is fully hydrated, it allows water uptake by diffusion until the equilibrium moisture content is achieved (Miano et al., 2018; Mandizvo & Odindo, 2019; Onwurafor et al., 2020).

3.7.2 Effect of steeping and sprouting times on the sprout length of Bambara groundnut green malts

The effect of steeping times on 36 and 48 h is shown in Figure 3.3. The sprout length increased with the means of 2.33 cm for the 36 h steeped seeds, and 48 h was 1.99 cm. The steeping times significantly ($p = 0.004$) affected the sprout development where 48 h steeped BGN seeds were shorter than the 36 h steeped seeds. The longer steeping regime slowed sprout growth and thus the reduction in sprout length in the 48 h steeped seeds. The longer steeping duration resulting in reduced sprout length was reported by Usansa et al. (2009). The shortest sprout length was observed in Thai rice malt cultivars (*Oryza sativa* l. Indica) steeped for 72 h, followed by 48 and 24 h. Also, Owusu-Mensah et al. (2011) noted that steeping at 48 h had longer sprouts than 72 h steeped rice grain. However, Mun et al. (2019), Wijngaard et al. (2005) and Chandraprabha & Sharon (2021) noted that longer steeping encourages an increase in sprout length resulting in higher water absorption in Korean red bean, buckwheat and millet, respectively. The increase in sprout length was attributed to steeping, which encourages respiration and energy metabolism (Lekjing & Venkatachalam, 2020; Neylon et al., 2020).

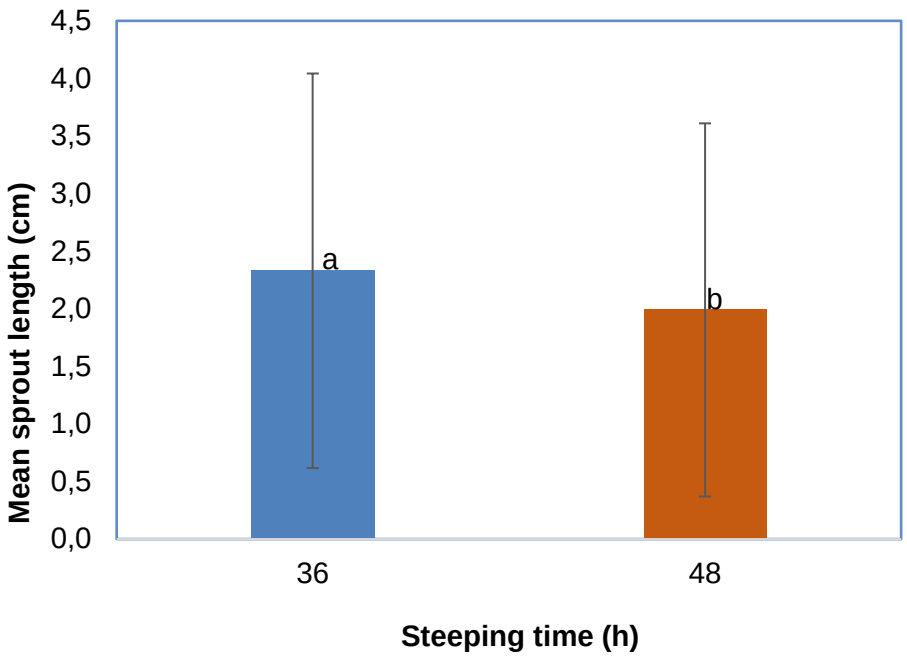


Figure 3.3: The effect of steeping times on the sprout length of Bambara groundnut seeds[1]. [1]Bars with different alphabets differ significantly (p = 0.004)

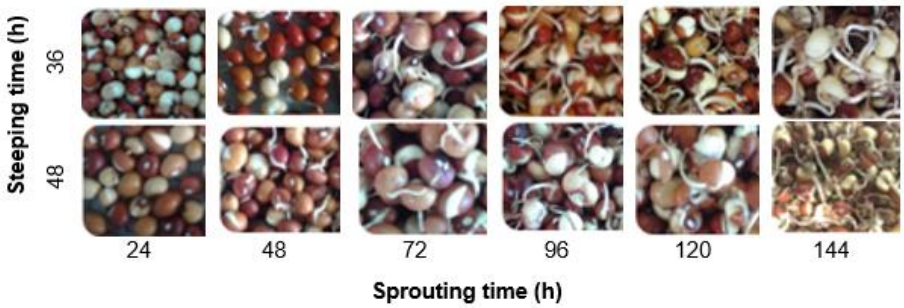
The rate at which respiration occurs is majorly dependent on the quantity of water uptake by the grains (Miano *et al.*, 2018; Neylon *et al.*, 2020; Mosher & Trantham, 2021). Then the seed modification is further encouraged by the gibberellic acids, a plant hormone that triggers movement from the embryo to the aleurone layer during steeping. (Xu *et al.*, 2020; Mosher & Trantham, 2021). Also, Pardeshi & Tayade (2013) noted that increased soaking period may result in anaerobic fermentation of soaked soybeans due to reserved foods limited availability causing the sprout lengths to be decreased. The sprout length differences showed the same trends suggesting that differences in steeping time in this study were primarily due to water immersion duration. In addition, it showed that the 48 h steeped BGN malt had high water uptake with shorter sprout length due to increased steeping time. This resulted in over-steeping, irregular and depressed germination, resulting in grain death known as induced water sensitivity (Hudson, 1986; Briggs, 1998; Guerrero, 2009).

The sprouting time from 0 up to 144 h affected the sprout length of the malted BGN seeds. The sprout length increased with the sprouting time, as shown in Figures 3.4a and b. The sprouted BGN seeds were significantly (p = 0.000) different, as illustrated in Figure 3.4c. The highest sprout length of 4.47 and 4.63 cm were observed for 36 and 48 h steeped

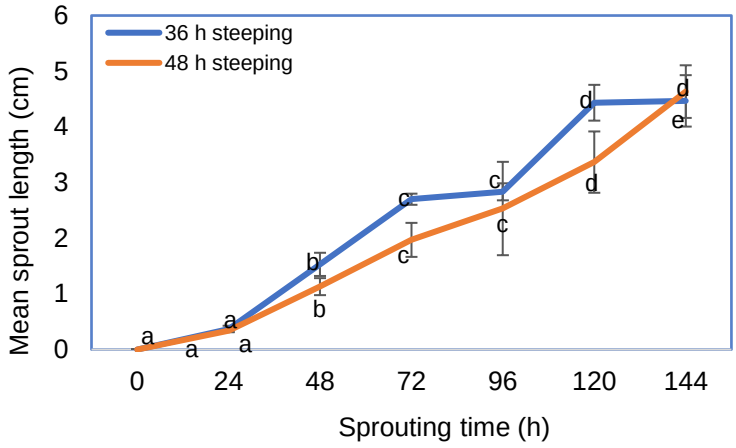
BGN malts at 144 h sprouting. However, the 36 h steeped seeds exhibited higher sprout lengths from 24 to 120 h of sprouting, but the sprout length of the 48 h steeped seeds was longer at 144 h. This study's differences in sprout length indicated that sprout length increased with sprouting time (Jiang *et al.*, 2013; Islam *et al.*, 2017). Likewise, Dahiya et al. (2018) noted that sprouting time affected the sprout length of grains. Similar observations were made by Murugkar & Jha (2009) and Devi et al. (2015) in soybean, BGN and cowpea seeds, where the sprouting duration affected their length.

The progressive sprout lengths increase from 24 to 144 h of sprouting for the two steeping regimes could be attributed to the successful modification of the seeds. The sprout length depends on the modification of the grains during sprouting. The gibberellic acid, a plant hormone, diffuses into the aleurone layer to signal the production of enzymes for stimulating acrospires growth (Guido & Moreira, 2013; Ikram *et al.*, 2021; Mosher & Trantham, 2021). The 36 h steeped seeds sprouted from 24 to 144 h had the highest sprout lengths. This result is similar to the study by Islam *et al.* (2022), where 24 h steeping time showed higher sprout length than 48 h attributed to prolonged soaking of 48 h, which inhibited sprout length caused by the accumulation of sucrose which is an inhibitor of α-amylase (Liu *et al.*, 2018a). Since steeping and availability of oxygen activate α- and β-amylase, which encourages increased acrospire length (Liu *et al.*, 2018a; Islam *et al.*, 2022), a proper steeping time is necessary for sprouting BGN seeds to give desirable bioactive compounds that can be used to develop functional food products. Steeping increased sprout length significantly because it encourages growth by breaking down starch into sugar via amylase (Lemmens *et al.*, 2019). In this study, steeping for 36 h and sprouting for 96 h would be an optimum condition for producing sprouted BGN to avoid the loss of bioactive components that may be beneficial to health.

(a)



(b)



(b)

(c)

Figure 3.4: Bambara groundnut (a) Sprout length changes from 24 to 144 h (b) Sprouting stages from 24 to 144 h (c) Increase in sprout length from 0 to 144 h sprouting time[1]

[1]Series with different alphabets differ significantly ($p < 0.005$)

3.7.3 Effect of steeping and sprouting time on the colour of Bambara groundnut malt

The CIE L*a*b* colour space coordinates of the steeped BGN seeds as affected by steeping times is in Table 3.1. The mean lightness (L*), redness (a*) and yellowness (b*) for 36 h steeping were 76.11, 2.93, and 11.45, respectively. The lightness (L*) redness (a*) and yellowness (b*) for 48 h were 75.60, 3.42, and 12.81, respectively. The chroma (C*) and hue angle (h^o) were 12.02 and 75.67^{o,} respectively. Based on steeping time, there was a significant ($p = 0.003$) difference in the lightness (L*) where the 36 h steeped BGN malt was lighter than the 48 h steeped seeds. The positive redness (a*) indicated that both steeping times exhibited redness in colour. However, the redness did not show a significant difference ($p = 0.157$) for the 36 and 48h steeping times. The steeped BGN malts differed significantly ($p = 0.002$) in yellowness (b*) between 36 and 48 h steeping times. The chroma (C*) and hue angle (h^o) were not significantly different for the 36 and 48 h steeping times.

Table 3.1 Effect of steeping time on colour characteristics of Bambara groundnut seeds [1]

	Steeping time (h)	
Colour parameters	**36**	**48**
Lightness (L*)	76.11 ± 6.02^a	75.60 ± 4.09^b
Redness (a*)	2.93 ± 2.27^a	3.42 ± 1.27^a
Yellowness (b*)	11.45 ± 2.05^a	12.81 ± 2.57^b
Chroma (C*)	12.02 ± 2.08^a	13.71 ± 10.40^a
Hue angle (h^o)	75.67 ± 10.40^a	71.10 ± 15.58^a

[1]Mean values ± standard deviation of triplicate determinations, mean values in the same row with different letters are significantly ($p \leq 0.05$) different. L*: Lightness; a*: Redness, b*: Yellowness

The steeping time increased the malts' redness (a*) and yellowness (b*). The increase in yellowness agrees with Bayram et al. (2004), where soybean was soaked to measure the soybean's colour change and the soaking water; the colour change was attributed to the degradation of red pigments into yellow colour. The rise in the malted BGN seeds yellowness could, however, be explained to be due to the leaching of plant pigments (water-soluble colour compounds) such as chlorophyll, xanthophyll, and carotene lost during steeping (Wood *et al.*, 2022). The decrease in lightness (L*) and increased redness (a*), respectively, were also due to pigment transfer from grain coat to endosperm and the

onset of modification of grains and legumes (Sofi *et al.*, 2020b). Likewise, changes in structure, disruption, the disintegration of molecules, and bond breakage decreased lightness by breaking down carbohydrates and proteins (Salem *et al.*, 2014; Odunmbaku *et al.*, 2018; Chinma *et al.*, 2021).

Although there was no significant difference in the chroma (C*) and hue angle (h^o), there was an indication of an increase in chroma (C*) and a decrease in hue angle (h^o) at an increase in steeping time. The chroma represents the colour intensity or strength of colour, starting from grey (Osuji *et al.*, 2020). The increased chroma due to the increase in steeping time suggested that the BGN malts had a less saturated hue angle (Dugulin *et al.*, 2021). The hue angle (h^o) is the quality attribute of colour defined as reddish, greenish and yellowish for 90, 180 and 270^o as perceived by human eyes (Pathare *et al.*, 2013; Osuji *et al.*, 2020). A higher hue angle represents a lesser yellow character; thus, the hue angle (h^o) for both steeping times were between 0° and 90°, where 0° represents the red colour, and 90° represents the yellow colour. Therefore, the hue angle of the BGN malts indicates that the BGN malts colour was reddish-yellow (Kortei and Akonor, 2015).

The differences in steeping time had an impact on the BGN malt colour quality, and this is in agreement with the study of Wood *et al.* (2022), where chickpeas exhibited darker seeds after steeping, attributed to the leaching of water-soluble phenolics consisting of yellow/red compounds such as anthocyanidin and flavanols. However, the leaching period is dependent on the soaking time and temperature (Bayram *et al.*, 2004; Wood *et al.*, 2022), leading to darker seeds (Turner *et al.*, 2019).

Sprouting time affected the lightness (L*) of the 36 and 48 h steeped BGN seeds ranging from 68.02 to 82.67 and 72.77 to 79.97, respectively, as shown in Table 3.2. The redness and yellowness were significantly ($p = 0.000$) different for the 36 and 48 h steeped BGN malt sprouted from 0 to 144 h. The redness (a*) and the yellowness (b*) for 36 h steeped BGN malts were 0.76 to 6.22 and 8.08 to 10.18, respectively (Table 3.1), while the 48 h steeped BGN malts were 1.70 to 5.00 and 8.18 to 13.14, respectively. The increase in sprouting time led to a reduction in lightness, making the malts darker. The malts positive values for redness (a*) and yellowness (b*) indicated that the BGN malts had more red and yellow pigments (Ramashia *et al.*, 2018). There was an increase in the redness (a*) and yellowness (b*) of the BGN malt as sprouting time increased from 24 to 144 h with a significant ($p = 0.000$) difference. Observed changes in the BGN malt colour can be attributed to the melanoidins (colour compounds associated with heat) due to the Maillard reaction during kilning (Osuji *et al.*, 2020).

Table 3.2 The effect of sprouting time on the colour of Bambara groundnut malts[1]

Sprouting Time (h)	L*	a*	b*	Chroma	Hue angle (h°)
36 h Steeping					
0	82.67 ± 0.43^{a}	0.76 ± 1.08^{a}	8.08 ± 0.88^{a}	8.16 ± 0.82^{a}	82.11 ± 4.32^{a}
24	81.59 ± 1.00^{b}	2.40 ± 1.63^{a}	10.05 ± 0.33^{b}	10.42 ± 0.34b	76.64 ± 9.01^{ab}
48	79.56 ± 0.08^{c}	1.63 ± 1.15^{a}	12.46 ± 0.86^{c}	12.60 ± 0.87^{cd}	82.53 ± 5.09^{a}
72	79.15 ± 0.07^{c}	1.45 ± 0.32^{a}	12.83 ± 0.51^{c}	12.91 ± 0.53^{cd}	83.57 ± 1.26^{a}
96	74.50 ± 0.54^{d}	2.49 ± 1.40^{a}	13.21 ± 1.13^{c}	13.50 ± 0.80^{ef}	79.06 ± 6.97^{ab}
120	67.27 ± 0.31^{e}	5.58 ± 0.71^{b}	13.33 ± 0.70^{c}	14.47 ± 0.58^{f}	67.27 ± 3.23^{bc}
144	68.02 ± 0.17^{e}	6.22 ± 1.83^{b}	10.18 ± 1.25^{b}	12.06 ± 0.56^{c}	58.51 ± 10.16^{c}
48 h Steeping					
0	79.97 ± 0.05^{a}	3.56 ± 0.31^{ab}c	8.18 ± 1.40^{a}	13.20 ± 2.22^{abc}	66.21 ± 3.65^{a}
24	80.14 ± 1.12^{a}	2.43 ± 0.52^{cd}	11.57 ± 1.04^{b}	6.55 ± 2.64^{ab}	54.15 ± 39.56^{a}
48	78.17 ± 0.26^{b}	1.70 ± 0.97^{d}	14.83 ± 0.70^{c}	3.96 ± 3.45^{a}	83.56 ± 3.40^{a}
72	74.43 ± 0.43^{d}	4.23 ± 0.68^{ab}	13.34 ± 0.44^{bc}	18.67 ± 6.02^{cd}	72.48 ± 2.12^{a}
96	75.49 ± 0.31^{c}	3.79 ± 1.55^{abc}	14.56 ± 2.70^{bc}	16.48 ± 11.27^{bcd}	74.78 ± 7.19^{a}
120	68.22 ± 0.74^{f}	5.00 ± 0.65^{a}	14.02 ± 1.14^{bc}	25.78 ± 6.75^{d}	70.23 ± 3.92^{a}
144	72.77 ± 0.07^{e}	3.22 ± 0.84^{bcd}	13.14 ± 2.38^{bc}	11.34 ± 5.41^{abc}	76.31 ± 1.57^{a}

[1]Mean values ± standard deviation of triplicate determinations, mean values in the same column within steeping time with different letters significantly differ ($p \leq 0.05$), L*: Lightness; a*: Redness, b*: Yellowness

The Maillard reaction is associated with the interaction of amino acids and reducing sugars in sprouted grains to produce Maillard Reaction Products (MRPs) during kilning due to the temperature and time of drying (Coghe et al., 2004; Runavot et al., 2011; Carvalho et al., 2016; Koren et al., 2019; Bettenhausen et al., 2020).

The chroma and hue angle of BGN malts for the 36 h steeping time ranged from 8.26 to 14.47^{o} and 58.51^{o} to 83.40^{o}, respectively, from 24 to 144 h of sprouting. The chroma and hue angle of BGN malts for the 48 h steeping ranged from 6.55 to 25.78 and 54.15^{o} to 83.56^{o,} respectively. There was a significant ($p = 0.000$) difference in chroma (C*) from 24 to 144 h of sprouting for the 36 and 48 h steeping times. However, there was no significant ($p = 0.139$) difference in the hue angle (h^{o}) from 24 to 144 h of sprouting. The hue angle is the primary colour characteristic that describes the red, green, blue, and yellow colours the human eye perceives (Ali *et al.*, 2008; Bhol & Sowriappan, 2014; Ramashia *et al.*, 2018). It measures an angle of 0° to 360° (0° and 360^{o} = red, 90° = yellow, 180° = green, and 270° = blue) (Ramashia *et al.*, 2018; Osuji *et al.*, 2020). The two steeping times hue angle were affected by sprouting with a range of 58.51^{o} to 83.57^{o} for 36 h steeped BGN malt and 54.15^{o} to 83.56^{o} for 48 h steeped BGN malt. The two steeping regimes' hue angles for the sprouted BGN malts were less than 90^{o}, indicating reddish-yellow as described in previous studies (Jha, 2010; Pathare *et al.*, 2013).

The changes in lightness, redness, yellowness, hue angle, and chroma of malted BGN in this studies could be attributed to differences in the steeping and sprouting times, producing different soluble sugars and protein content (amino acids) (Edney & Izydorczyk, 2003; Chung et al., 2012; Setia, 2019). The changes in the colour coordinates of the malted BGN correlates with sprouted mung bean flour and malted sorghum-soy becoming darker with the increase in germination time due to the enzymatic hydrolysis during germination (Liu *et al.*, 2018b; Asuk *et al.*, 2020). Also, the study of BGN based on colour has shown that seed coat colour affected seed germination attributed to the impact of the hydrolytic potential of the BGN and Maillard reaction during drying treatment (Gqaleni, 2014; Harris *et al.*, 2018). The colour changes for the sprouted BGN seeds are as shown in Figure 3.5.

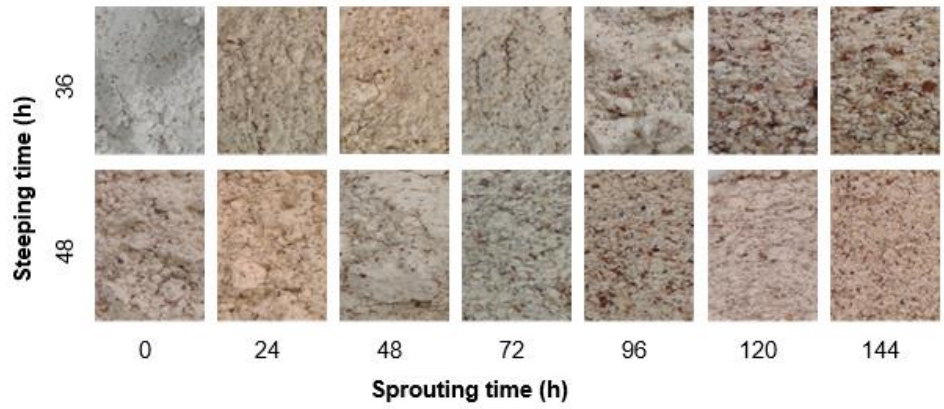


Figure 3.5: Effect of sprouting time on colour characteristics of Bambara groundnut malts produced from 36 and 48 h steeping regimes

The colour formation during malt processing significantly impacts the appearance and acceptability of food malt products (Olamiti *et al.*, 2020; Dugulin *et al.*, 2021). Lightness (L*), redness (a*), and yellowness (b*), chroma, and hue angle are important indications of the quality of the product and market worth (Melgosa et al., 2004; Simons, 2018). Recently, consumers have been asking for natural food colours in food production, which has increased the demand for malted grains as colourants in food production (kaemmej, 2020, 2021; Parr *et al.*, 2021). The BGN malts colour characteristics of reddish-yellow in this study is a colour combination that is important in food processing industries due to its ability to grab attention, evoke the tastebuds and stimulate appetite (Bamforth, 2016; Ranaweera *et al.*, 2020). Hence, the malts produced from BGN seeds could be a natural source of colour in food production to enhance food colour.

3.7.4 Effect of steeping and sprouting time on the pH of Bambara groundnut malt

The mean pH of the 36 and 48 steeped BGN seeds was 6.11 and 6.13, respectively. The 48 h steeped BGN seeds pH values were significantly ($p = 0.001$) higher than the 36 h steeped seeds, as shown in Figure 3.6. The increase in pH exhibited could be attributed to increasing hydrogen ion content due to the biological activity of the carbohydrates and other food nutrients to produce organic acids (South, 1996; Obadina *et al.*, 2008).

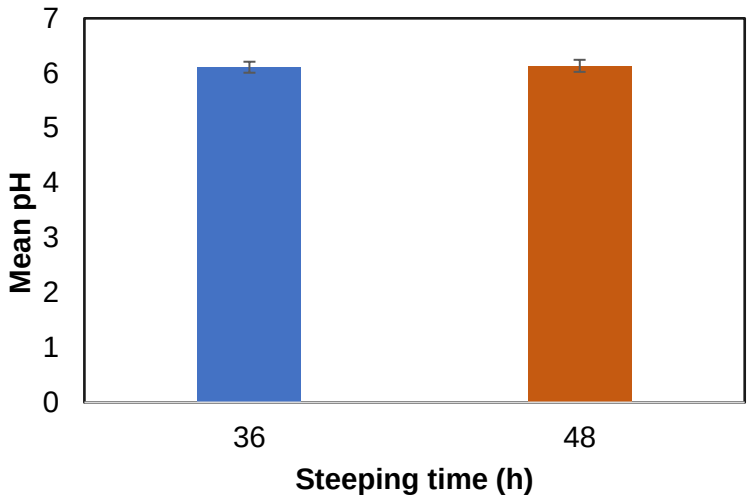


Figure 3.6: Effect of steeping time on Bambara groundnut seeds pH

Sprouting from 24 to 144 h showed a significant (p = 0.000) difference for both the steeping regimes. The pH for 36 h steeped BGN malt ranged from 5.94 to 6.21, while the 48 h steeped BGN malt values ranged from 5.95 to 6.22, as shown in Figure 3.7. During sprouting, the pH decrease could be due to the conversion of free fatty acids for energy generation by the lipase activity acting as triacylglycerol (Ferreira *et al.*, 2019; Atudorei *et al.*, 2021). It was also suggested that the decrease in pH of sprouted finger millet flour might be due to the production of organic acids during the sprouting time (Nefale & Mashau, 2018). Similar to this work are the studies on germinated maize and horse gram flour, where germination resulted in a reduction in pH (Adedeji et al., 2014; Handa et al., 2017). Handa et al. (2017) also reported that the decrease in pH was attributed to the reduction in enzymes' secretion that hydrolyses complex organic molecules such as phytic acid and protein into simpler acidic compounds such as phosphate and amino acids, respectively.

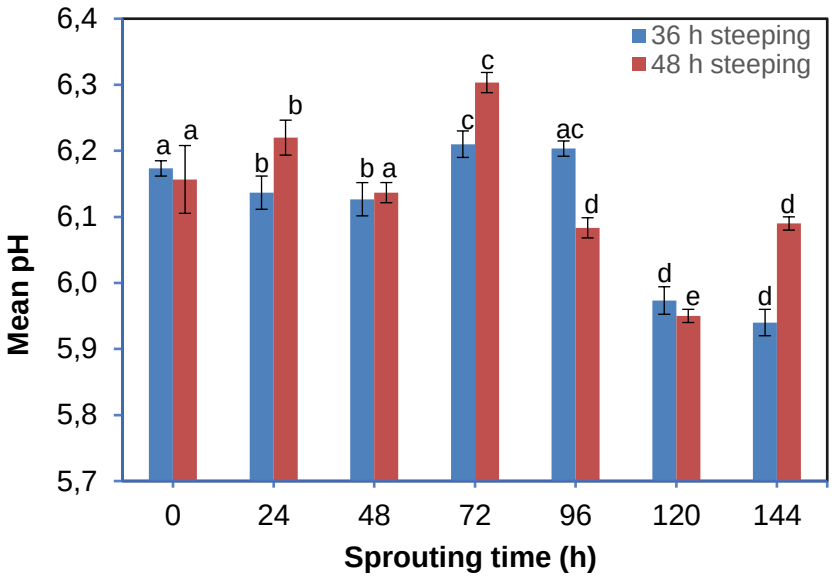


Figure 3.7: Effect of sprouting time on pH of Bambara groundnut malt produced from seeds sprouted for 36 and 48 hours [1]

[1]Bars with different alphabets differ significantly ($p < 0.05$).

3.7.5 Effect of steeping and sprouting time on the protein content of Bambara groundnut malt

The 36 h steeped BGN malt mean protein was19.98%, and the 48 h steeped BGN was 20.55%, as shown in Figure 3.8. Based on the Kruskal Wallis, protein distribution is the same across the steeping time (h), thus showing no significant difference. The same result was observed by Judith Kanensi *et al.* (2011) and EL-Suhaibani et al. (2020), where there were no significant differences in the protein contents of amaranth and goat pea (*Securigera securidaca* L.) subjected to varying steeping time. However, Widjajaseputra *et al.* (2019) and Handa *et al.* (2017); reported that steeping time increases the protein content in mung bean and amaranth grains. The increase is attributed to the change in the starch, water, and lipids components in the grains during steeping, which may have altered the protein's proportion on dry weight matter (Akpapunam et al., 1996; Asouzu & Umerah, 2020). The increase in the protein content could also be due to increased free amino acids, peptides and the rise in non-protein nitrogenous contents during steeping (Murugkar & Jha, 2009; Murugkar, 2014; Dattatray et al., 2020). These contrary results were attributable to different factors, including species and variety, seed availability, and environmental conditions by Okoth *et al.* (2011).

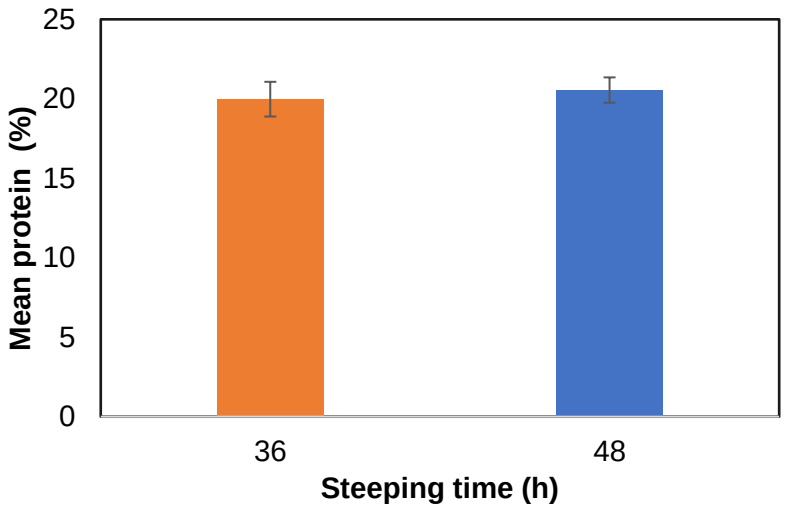


Figure 3.8: The distribution of protein across categories of steeping time

The crude protein based on the sprouting time showed no significant difference from 0 to 144 as analysed by Kruskal Wallis in Figure 3.9. The result is similar to the studies on germinated legumes, mung beans, goat pea, and light brown speckled kidney beans (Mbithi *et al.*, 2001; EL-Suhaibani *et al.*, 2020). Several studies have shown that sprouting increases the protein content of lupin, peas, chickpea, moth beans, soya beans, and mung beans (Bau *et al.*, 1997; Urbano *et al.*, 2005; Shah *et al.*, 2011; Medhe *et al.*, 2019; Xu *et al.*, 2019; Onwurafor *et al.*, 2020; Winarsi *et al.*, 2020; Kumar *et al.*, 2021). Researchers have also observed lower protein content in sprouted legumes, resulting from seed types and conditions of sprouting (Gulewicz et al., 2008; Dipnaik & Bathere, 2017; Medhe et al., 2019; Kumar et al., 2021). The change in protein content has been attributed to the interaction between protein degradation and biosynthesis as steeping and germination time increases (Onwuka & Ezemba, 2013; Handa et al., 2017; Benincasa et al., 2019; EL-Suhaibani et al., 2020). Also, legumes' protein content depends on the type of legume seeds and processing conditions such as steeping and sprouting (Trugo *et al.*, 1999; Aguilera *et al.*, 2014; Nkhata *et al.*, 2018; Lemmens *et al.*, 2019).

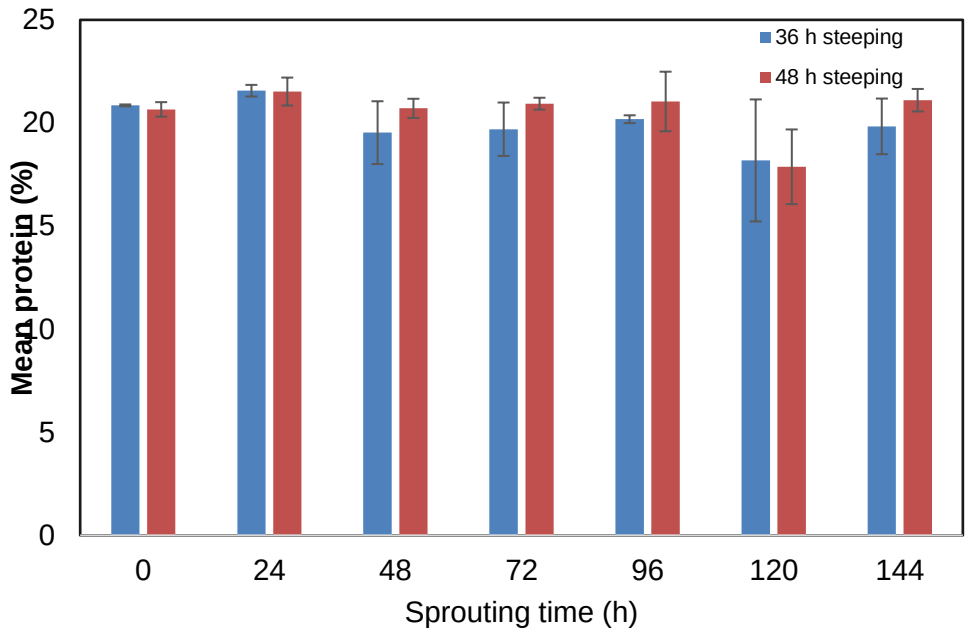


Figure 3.9: Effect of sprouting time on protein content of Bambara groundnut malt produced from seeds sprouted for 36 and 48 hours

3.8 Effect of Steeping and Sprouting Time on α- and β- Amylase Activities of Bambara groundnut malt

Steeping at 36 and 48 h showed a significant ($p = 0.000$) effect on the α- and β-amylase activities of the malted BGN seeds, as shown in Table 3.3. Steeping at 36 h had mean α-amylase activities of 0.14 CU/g, while β-amylase activities were 0.21 BU/g. While steeping at 48 h had mean α-amylase activities of 0.17 CU/g and β - amylase of 0.22 BU /g. The 48 h steeped BGN malt has higher α and β-amylase activities than the 36 h steeped BGN malt. The difference is in agreement with the research on the amylase activities of mung bean (*Phaseolus aureus*), cowpea (*Vigna catjang*), lentil (*Lens culinaris*), and chickpea (*Cicer arietinum*) (Ghavidel & Davoodi, 2011). The increase in amylase activity with an increase in steeping time is due to enzymes' activation during steeping and the penetration of the gibberellic acid by diffusion to the aleurone layers to prompt enzyme synthesis (Usansa *et al.*, 2009; Tuan *et al.*, 2019; Xu *et al.*, 2020; Mosher & Trantham, 2021). Based on steeping time, BGN seeds steeped at 48 could give optimum α and β–amylase activities.

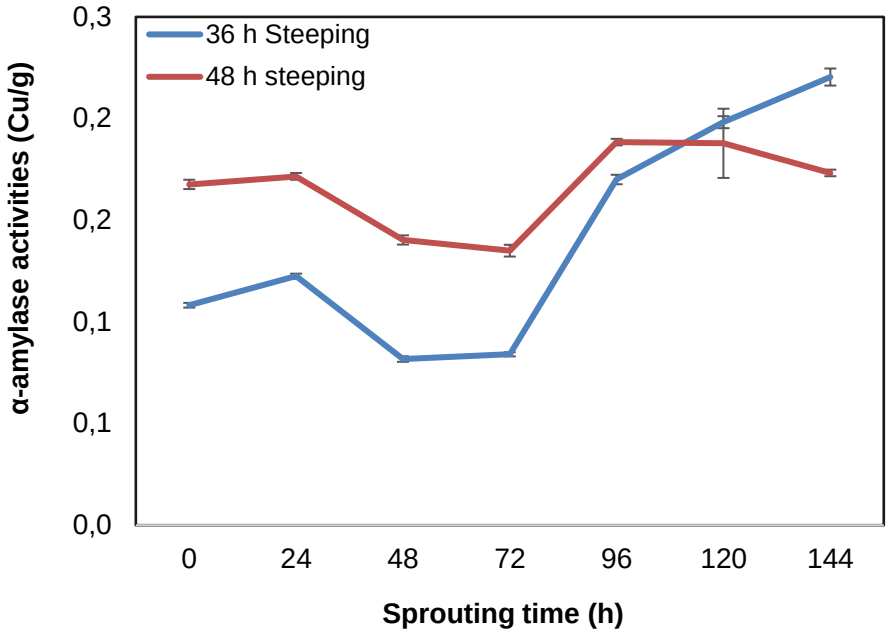
Table 3.3 α and β-amylase activities as affected by steeping time[1]

	Steeping time (h)	
Amylase activities	**36**	**48**
Alpha amylase (CU/g)	0.14 ± 0.05[a]	0.17 ± 0.02[b]
Beta amylase (BU/g)	0.21 ± 0.07[a]	0.22 ± 0.03[b]

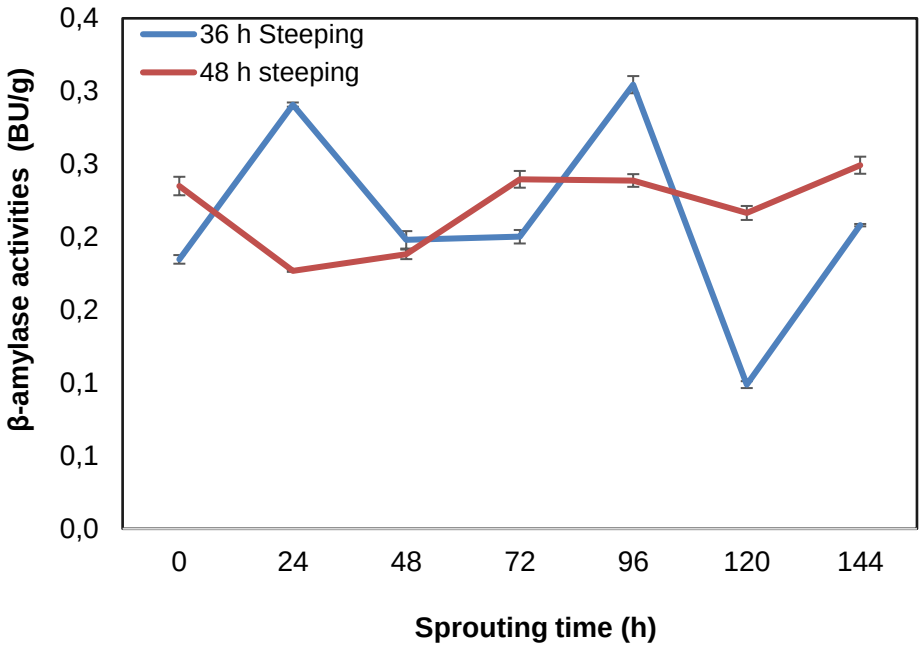
[1]Mean values ± standard deviation of triplicate determinations. Mean in the same row followed by different letters are significantly ($p < 0.05$) different

There was a significant ($p = 0.000$) difference based on sprouting time for the α- and β-amylase activities of BGN malt. The mean α-amylase activities for the 36 steeped malted BGN ranged from 0.11 to 0.22 Cu/g, while the 48 h steeped seeds ranged from 0.17 to 0.19 Cu/g shown in Figure 3.10a. The β-amylase activities ranged from 0.18 to 0.30 Bu/g and 0.18 to 0.25 BU/g for the 36 and 48 h steeped seeds, respectively in Figure 3.10b. There was increase in the activities of the α- and β-amylases as sprouting time increased, but the increase was not generally progressive. The increase in the α- and β-amylase activities in BGN malted seeds agreed with the results of enzyme activities of germinated mung bean (*Phaseolus aureus*), cowpea (*Vigna catjang*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*) and adzuki bean (*Vigna angularis*) increasing with increase in sprouting time (Uriyo, 2001; Ghavidel *et al.*, 2011; Hung *et al.*, 2020b, a; Chiu, 2021).

The increase in amylase activity resulted from the seeds absorbing water while steeping, subsequently mobilising their dormant reserve (Liu & Hou, 2018; Tuan *et al.*, 2019). The absorbed water then stimulates the embryo to produce gibberellic acid, which influences seed growth and developmental processes, including dormancy and germination of the seeds (Yousif & Evans, 2020). The gibberellic acid then diffuses to the aleurone layer and starts a flow resulting in the synthesis of α and β–amylase (Ghavidel & Davoodi, 2011; Ali & Elozeiri, 2017; Guzmán-Ortiz et al., 2019; Yousif & Evans, 2020). However, based on sprouting times, the 36 h steeping and 96 h sprouting times could be regarded as an optimum to produce an amylase rich malted BGN seed.



(a)



(b)

Figure 3.10: Effect of sprouting time on (a) α-amylase activities and (b) β-amylase activities of Bambara groundnut

3.9 Effect of Steeping and Sprouting Time on Total Polyphenol Content of Bambara groundnut Malt

There was a total polyphenols concentration of 0.92 mg GAE/g for 36 h and 0.99 mgGAE/g for 48 h steeping, as illustrated in Figure 3.11. There was an increase in total polyphenols from the 36 h steeped BGN seeds to the 48 h steeped seeds. The increase indicated that polyphenols content improved with an increase in steeping times of BGN seeds. The increase is in agreement with the study of beans and pinto beans that total polyphenolic compounds increase with soaking (Akillioglu & Karakaya, 2010).

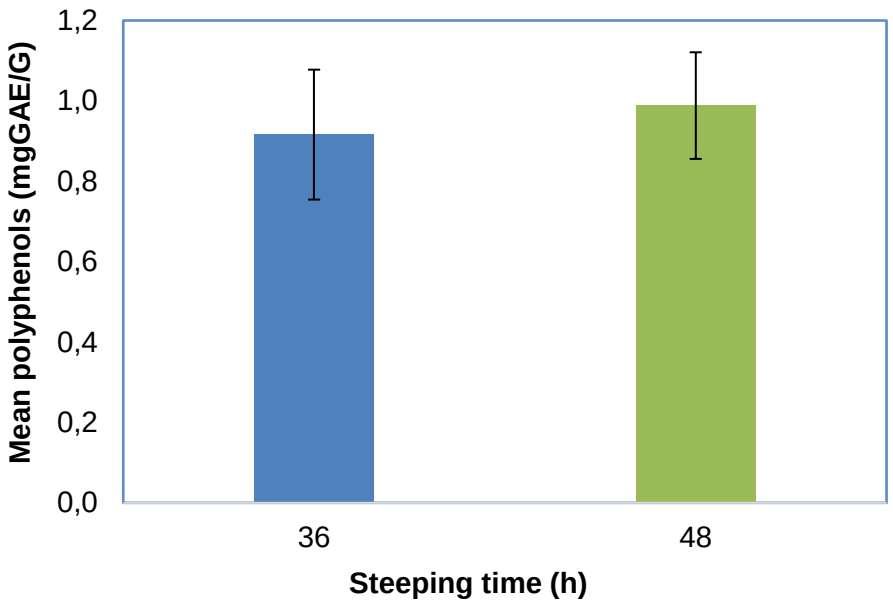


Figure 3.11: Total Polyphenols Content (mg GAE/g) of BGN seeds based on steeping time

The increase is attributed to polyphenols solubilisation due to water uptake during steeping (López-Amorós et al., 2006; Duodu, 2014; Mamilla & Mishra, 2017). However, most studies have shown that total polyphenols are reduced when legumes are steeped, depending on the soaking conditions (time and temperatures) and legume varieties (Xu & Chang, 2008b; Mazahib et al., 2013; Tajoddin et al., 2014; Eshraq et al., 2016; Adebiyi et al., 2019; Mba et al., 2019). Barimalaa & Anoghalu (1997), in their study, however, noted that cold-soaking overnight had a minimal effect on the rate of polyphenols loss in BGN seeds.

Sprouting time had a significant difference ($p = 0.000$) on the total polyphenol content of sprouted BGN seeds, as shown in Figure 3.12. The total polyphenol increased

with sprouting time, with the highest concentration at 144 h (1.22 mgGAE/g) sprouting for the 36 h steeping while 48 h steeping was at the 120 h (1.10 mgGAE/g) sprouting. The increase in total polyphenolic concentration indicated an improvement in total polyphenols based on sprouting time (Naveena and Bhaskarachary, 2013).

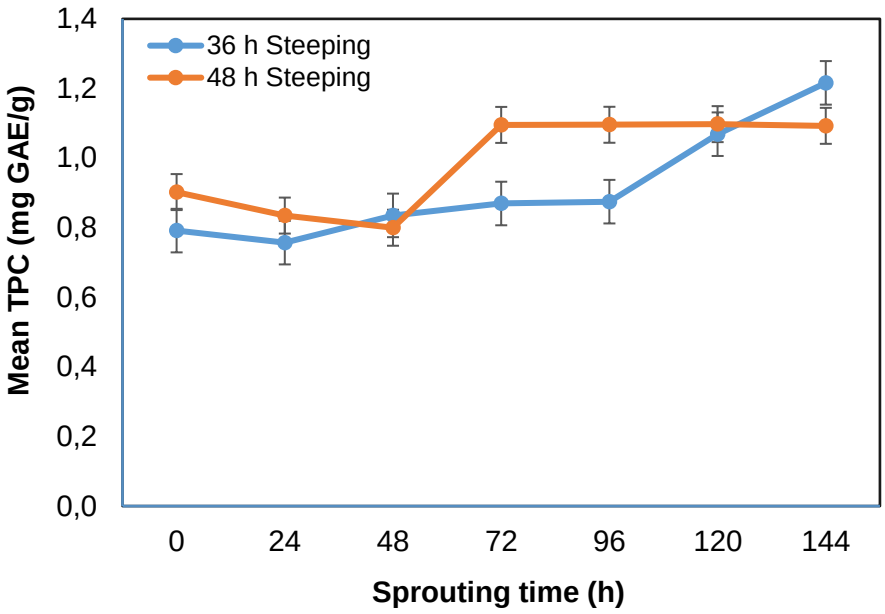


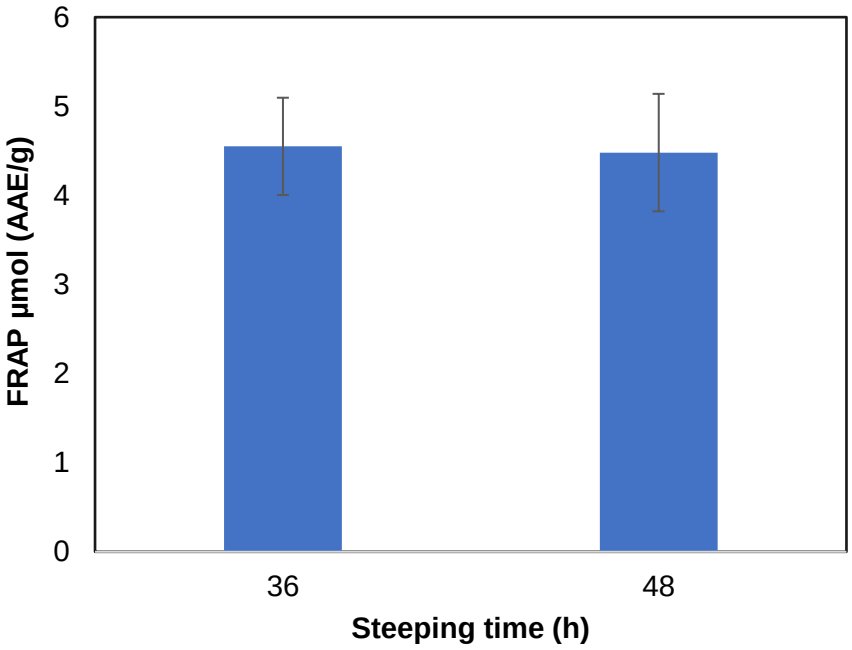
Figure 3.12: Total polyphenolic contents (mg GAE/g) with sprouting time

Total polyphenol contents increased significantly ($p \leq 0.05$) with an increase in steeping and sprouting time. The relative increase in total polyphenol contents during sprouting was reported by Xue et al. (2016) and Khang et al. (2016) for mung beans, black beans, and soybeans, Salem et al. (2014), and Yeo & Shahidi (2017) for faba beans, chickpea seeds, lentils seeds, fenugreek seeds and Dueñas et al. (2009) for lupine seeds. An increase in phenolic compounds was observed in soybean and mung beans with an increase in germination time (Naveena & Bhaskarachary, 2013; Hou et al., 2019; Liu et al., 2020). The increase might be attributed to the formation of phenol compounds during sprouting time. The increase in polyphenols could also be due to condensed tannin's solubilisation during seed soaking (Salem *et al.*, 2014; Saleh *et al.*, 2019; Schendel, 2019). Furthermore, the increase in polyphenols has been attributed to the link between enzyme activity and water availability during malting (Winarsi *et al.*, 2020). The absorption of water activates the dormant enzymes to stimulate growth. The stored enzymes are hydrolysed,

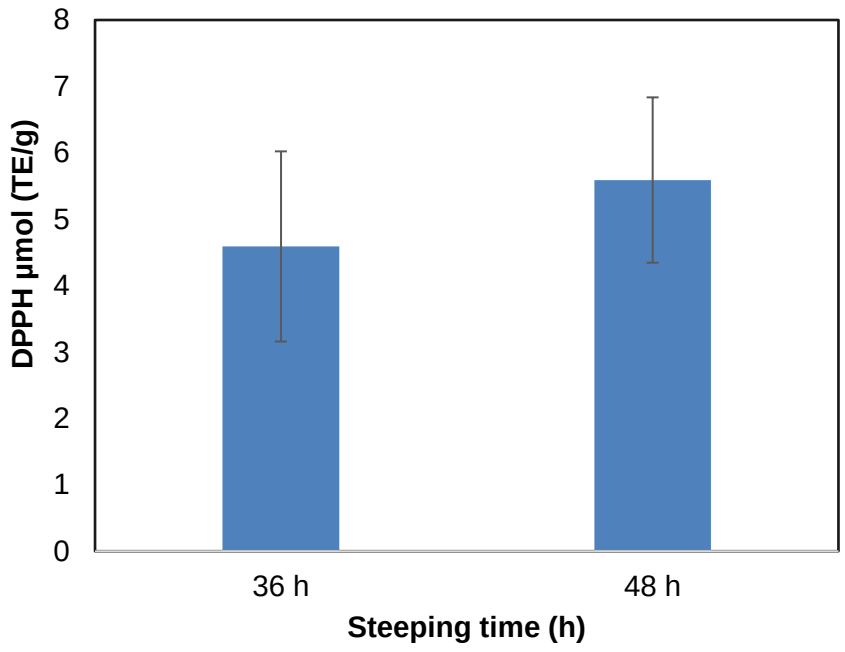
making the enzyme-substrate produce new products (phenolic). The bound phenolic compounds become free by activating endogenous enzymes during germination (Gharachorloo *et al.*, 2013; Sofi *et al.*, 2020b). Similar findings were reported by Sofi et al. (2020b) in germinated chickpea flour, Borges-Martínez et al. (2021) in pea and black bean, Fouad & Rehab (2015) in lentil sprouts, and Gharachorloo et al. (2013) in four legumes. However, the increase in total polyphenols as sprouting time increases was attributed to the phenolic composition changes caused by the endogenous enzyme activation and seeds' biochemical metabolism during the sprouting process (Guo *et al.*, 2012; Winarsi *et al.*, 2021). The increase in total polyphenolic contents in this study during steeping and sprouting times showed BGN malt is an antioxidant-rich product and was improved with steeping and sprouting time, and this could, however, be beneficial for consumers with oxidative stress associated diseases (Nyau *et al.*, 2015; Winarsi *et al.*, 2021). These findings indicated that an increase in total polyphenols of sprouted BGN has the potential for use in the nutraceutical industry to offer some health benefits to consumers.

3.10 Effect of Steeping and Sprouting Time on the Antioxidant Activities of Bambara groundnut malt

Steeping BGN seeds for 36 h had a mean antioxidant activity of 4.55 µmol (AAE/g) for FRAP and 4.59 µmol (TE/g) for DPPH assays, respectively. For both assays' 48 h steeping antioxidant activities was higher than the 36 h BGN steeped seeds. There was a significant ($p = 0.000$) difference between FRAP and DPPH antioxidant concentration in the 36 and 48 steeping times, as shown in Figure 3.13. The changes in the FRAP and DPPH assayed antioxidant concentrations is attributed to leakage of antioxidant compounds in soaking water (Salem *et al.*, 2014; Garretson *et al.*, 2018). Also, soaking for longer times resulted in higher biochemical metabolism of the seeds by releasing more phenolic compounds resulting in increased antioxidant activity, as shown in 48 h steeped BGN seeds (Mohammed *et al.*, 2017). Also, Erba et al. (2019) hypothesised that soaking water remaining from the seed may have extracted the soluble free and linked phenolic, thus increasing the antioxidant capacity.



(a)



(b)

Figure 3.13: Effect of soaking time on the antioxidant activities of Bambara groundnut (a) FRAP assay and (b) DPPH assay

Sprouting from 24 to 144 h for both 36 and 48 h steeped BGN malt FRAP antioxidant did not show a significant difference, as shown in Table 3.4. However, the 48 h steeped seeds sprouted for 120 h had the highest antioxidant activity. There was a significant ($p = 0.005$) increase from 24 to 144 h of sprouting in DPPH radical scavenging antioxidants concentration for the 36 and 48 h steeped BGN malt difference.

Table 3.4 Sprouted BGN seeds antioxidant activities

		Steeping time (h)[1]	
Antioxidant assay	**Sprouting time (h)**	**36**	**48**
FRAP umol (AAE/g)	0	5.14 ± 0.38^{a}	3.90 ± 0.17^{a}
	24	5.12 ± 0.38^{a}	3.80 ± 0.16^{a}
	48	4.53 ± 0.06^{b}	3.80 ± 0.19^{a}
	72	4.47 ± 0.24^{b}	4.93 ± 0.48^{bc}
	96	3.60 ± 0.02^{c}	4.60 ± 0.17^{b}
	120	4.30 ± 0.10^{b}	5.21 ± 0.09^{c}
	144	4.72 ± 0.13^{ab}	5.14 ± 0.55^{bc}
DPPH umol (TE/g)	0	3.68 ± 1.11^{a}	4.57 ± 0.99^{a}
	24	3.52 ± 0.80^{a}	4.47 ± 0.80^{a}
	48	3.75 ± 1.52^{a}	5.04 ± 0.21^{a}
	72	4.38 ± 1.66^{ab}	5.44 ± 1.64^{a}
	96	4.94 ± 0.98^{ab}	6.11 ± 1.06^{a}
	120	6.25 ± 0.12^{b}	7.44 ± 0.41^{b}
	144	5.62 ± 1.59^{ab}	6.08 ± 0.19^{ab}

[1]Values are mean ± standard deviation of triplicate values, mean values in the same column followed by different letters are significantly ($p < 0.05$) different FRAP and DPPH activities, respectively

The increase in antioxidants could be due to the activation of the natural endogenous antioxidants that occur in legumes during sprouting (Gharachorloo *et al.*, 2013; Fouad & Rehab, 2015b). However, the increase and modification of antioxidants in legumes depends on grain types and malting conditions (Fouad & Rehab, 2015b). Research on the malting process of legumes such as soybeans, pea, mung beans, lentils, cowpea, jack bean, dolichol and mucuna showed that an increase in sprouting time increased antioxidant

activities (López-Amorós *et al.*, 2006; Guo *et al.*, 2012; Aguilera *et al.*, 2013; Khang *et al.*, 2016; Borges-Martínez *et al.*, 2021).

The result of the antioxidant activities using FRAP and DPPH assays in this study indicated that steeping and sprouting times increased antioxidant activities in BGN seeds. However, the DPPH free radical scavenging antioxidant activities were higher, suggesting that they contain components that can scavenge free radicals to increase antioxidant activities (Nyau *et al.*, 2015). Increased antioxidant activity in BGN malt was due to the release of phenolic compounds bound to the cell structure during steeping and sprouting times (Pejin *et al.*, 2009). Steeped BGN seeds at 48 h, sprouting for 120 h and assayed using the DPPH gave antioxidant-rich BGN malt. The higher antioxidants in the 48 h steeped and 120 h sprouted seeds exhibited the potential of BGN as a legume with incredible beneficial properties for food and industrial applications.

3.11 Conclusions

This study showed that steeping and sprouting times affected the physicochemical characteristics of BGN seeds. The colour of BGN was reddish-yellow, which is a desirable colour combination to improve or enhance the colour of food produced. The malting process significantly affected the amylase enzyme activity of BGN seeds. The steeping and sprouting processes increased the amylase activities of the BGN malt for both 36 and 48 h steeping times. However, an amylase-rich BGN malt could be produced by steeping for 36 h and sprouting for 96 h. In addition, total polyphenols and antioxidants improved during the steeping and sprouting processes. Steeped BGN seeds for 48 hours, sprouted for 120 hours resulted in an antioxidant-rich BGN malt that could have nutraceuticals benefits. Bambara groundnut malt under properly controlled malting conditions could be perfect for new food production, like barley malt. Malt products are a good source of colour, amylase and antioxidant, and food industries rely on them for food production (Edney & Izydorczyk, 2003; Singh et al., 2017a, b). Furthermore, BGN is a climate change crop (Feldman *et al.*, 2019) that could ease the demand for malt uses and add beneficial properties in food and industrial applications.

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CHAPTER FOUR
EFFECT OF STEEPING AND SPROUTING TIMES ON THE METABOLITES OF MALTED BAMBARA GROUNDNUT

Abstract

Recently, metabolomics has been considered an effective tool for explaining the information on metabolites. Its use in food analysis is based on the application of omics technologies to study the food micro-structure and nutrient distribution. Metabolite profiling of cereals and legumes have been carried out to investigate their low molecular metabolites. However, there is nothing done on the metabolites of Bambara groundnut (BGN) based on the steeping and sprouting times. Bambara groundnut seeds were steeped for 36 and 48 h, then sprouted from 24 to 144 h. The sprouted BGN were taken every 24 h up to 144 h during sprouting of the seeds and were analysed for fatty acid methyl esters, hydrocarbons, fatty alcohols, sugars, acids, amino acids, and volatile components. Metabolite profiling method based on capillary gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionisation detection (GC-FID) was employed to study the time-dependent metabolic changes during the malting process. Changes in the metabolic constituents during the malting process of BGN seeds were affected by steeping and sprouting times. Heatmap and agglomerative hierarchical clustering analysis also showed differences in the 36 and 48 h steeped BGN seeds sprouted from 0-144 h. The metabolites showed different levels of fatty acid methyl esters (FAME), sugars and organic acids, amino acids, and volatiles in the sprouted BGN seeds. However, FAME was not significantly different among the different steeping times. Linoleic acids were high after 48 and 96 h sprouting for the two steeping times. Sugars and acids decreased as steeping time increased except for myo-inositol, which increased. Sugars, acids, and sugar alcohol were significantly ($p \leq 0.05$) different across the sprouting times for the two steeping regimes. The two steeping regimes differed significantly in the amino acid concentration. The essential amino acids lysine, isoleucine, methionine, threonine, valine, phenylalanine, and leucine increased with sprouting time. However, an increase in steeping time reduced the volatile concentration. There were changes in volatiles concentration throughout the sprouting process. Ten volatiles were identified in the raw BGN seeds; nine volatiles in steeped BGN seeds (36 and 48 h) while 21 and 14 volatiles were detected in the sprouted 36 and 48 h steeped BGN, respectively, from 0 to 144 sprouting time. The volatiles detected consisted of hydrocarbons, organic, alkanes, ketones, and aromatics compounds. Ten volatiles were identified in the raw BGN seeds, 17 volatiles in the 36 and 48 h steeped BGN seeds, while the sprouted 36 and 48 h steeped seeds had 21 and 14 volatiles. The volatile

furan, 2 pentyl characterised by an earthy, beany, nutty pungent aroma, was common in the sprouted BGN seeds. However, the volatile could be optimised by steeping for 48 h and sprouting up to 120 h.

4.1 Introduction

Metabolomics, the combination of metabolite and genomics (Nikolau, 2007)**,** is a popular modern approach for detecting large numbers of low molecular mass compounds. It has been applied successfully in drug discovery, food science and biological systems studies (Beleggia *et al.*, 2013; Aggio *et al.*, 2014). Metabolomics has been used as a suitable tool for investigating metabolite changes in germinating/malting cereals and legumes (Jom *et al.*, 2011; Frank & Engel, 2013; Bertram & Jakobsen, 2018). The small molecule metabolites or chemicals in cells and organisms can be detected, identified and quantified by metabolomics (Wishart, 2008; Wood, 2021). Metabolomics is considered an important means of studying metabolites in food processing, agriculture, pharmaceutical companies and understanding the science of our immediate environment (Cevallos-Cevallos *et al.*, 2009; Roessner & Bowne, 2009; Cevallos-Cevallos & Reyes-De-Corcuera, 2012).

Food metabolomics (foodomics) is applied to food systems, comprising food resources, food processing and diet for humans. In recent years, the use of food metabolomics has increased because industrial food production is related to nutrition and human health (Kim *et al.*, 2016; Adebo *et al.*, 2021). The application of metabolomics in food science starts from the farm produce, food processing, safety, toxicology, microbiology to food quality (García-Cañas *et al.*, 2012; Capozzi & Bordoni, 2013). Foodomics has been made possible by sample preparation, extraction (polar and non-polar), fractionation, detection, identification, and data analysis (Herrero *et al.*, 2012; Ibáñez *et al.*, 2012; Cifuentes, 2013, 2017; Schasteen, 2016; Ferranti, 2018). In addition, the analysis can be targeted or non-targeted metabolite profiling (Roessner & Bowne, 2009; Vinayavekhin & Saghatelian, 2010).

Techniques such as capillary gas chromatography-mass spectrometry (GC-MS), gas chromatography-flame ionisation detector (GC-FID), high-performance-liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) are commonly used in foodomics (Castro-Puyana *et al.*, 2013; Ibáñez *et al.*, 2013; Oms-Oliu *et al.*, 2013). The metabolite profiling of the sprouting mung beans (*Vigna radiata*) and germination of brown rice was carried out using GC-MS to investigate time-dependent metabolic changes during the malting process (Shu *et al.*, 2008; Jom, 2012). Ultra-high-performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-TOF-MS) was used to profile the polyphenols in mung beans sprout during the germination process (Tang *et al.*, 2014a). GC-MS and LC-MS were used to

establish the effects of sprouting on the nutritional quality of soybean sprouts (Gu *et al.*, 2017).

The metabolomics analysis of germinated soybean, fava beans, black gram, chickpea, cowpea, and mung beans sprouts showed that sprouting enhanced their nutritional and functional properties. Also, healthy food products can be produced from sprouts (Tang *et al.*, 2014b; Limwiwattana *et al.*, 2016; Mekky *et al.*, 2020; Farag *et al.*, 2021a,b). Furthermore, the investigation on the effect of sprouting on the phenolic phytochemicals of the red Bambara groundnut (BGN) seeds by HPLC-PDA-ESI-MS showed that changes in the sprouted BGN were positive and beneficial to health (Nyau *et al.*, 2017). Unfortunately, BGN is still underutilised because it is mostly farmed locally by women in the rural areas of Africa where it originated (Mayes *et al.*, 2015, 2019; Mabhaudhi *et al.,* 2016; Halimi *et al.*, 2020). However, BGN has shown good health-promoting characteristics based on its processing methods. Soaking, roasting, boiling, autoclaving, milling, sprouting, and malting processes have been applied to BGN to harness its high nutritional content (Omoikhoje, 2008; Abiodun & Adepeju, 2011; Mazahib *et al.*, 2013; Ndidi *et al.*, 2014; Asia, 2020).

Malting (steeping, sprouting and drying) has been shown to increase, improve and modify bioactive components of cereals and legumes (Giebel, 2015; Khang *et al.*, 2016; Duodu & Apea-Bah, 2017; Wani & Jain, 2018; Guo, 2019). The previous chapter showed improved changes in the physicochemical, enzyme and antioxidant activities of malted BGN seeds subjected to different steeping and sprouting times. However, unlike legumes such as mung bean and soybeans, the metabolite of malted BGN during malting is yet to be profiled. Hence, the need to identify and quantify the bioactive components of malted BGN seeds to estimate the effect of steeping and sprouting times.

This study aimed to profile the metabolites of malted BGN seeds by analysing the broad spectrum of low molecular weight metabolites from the range of chemical classes present, identifying and quantifying the metabolite components based on steeping and sprouting times.

4.2 Materials and Methods

4.2.1 Source of materials, reagents, and equipment

The sprouted 36 and 48 h steeped BGN seeds produced in Chapter 3 were used as samples for metabolite profiling. Chemicals and reagents were of analytical standards. All other equipment used was in the Department of Food Science and Technology and Central Analytical Facilities (CAF) - Stellenbosch University, Cape Town, South Africa.

The equipment and instruments used in this study were the ten trays dehydrator (Model 1069616 Excalibur, Sacramento, CA, USA), Waring Laboratory Science blender

model 7009G (Waring Laboratory Science, CT, USA), a water bath, incubator, gas chromatography-mass spectrometric coupled with Flame Ionization Detector (GC-FID). In addition, gas-chromatography (Trace1300, Thermo Scientific) was coupled to Agilent TSQ8000 mass spectrometer (Thermo Scientific). Figure 4.1 outlines the process and analyses carried out in this chapter.

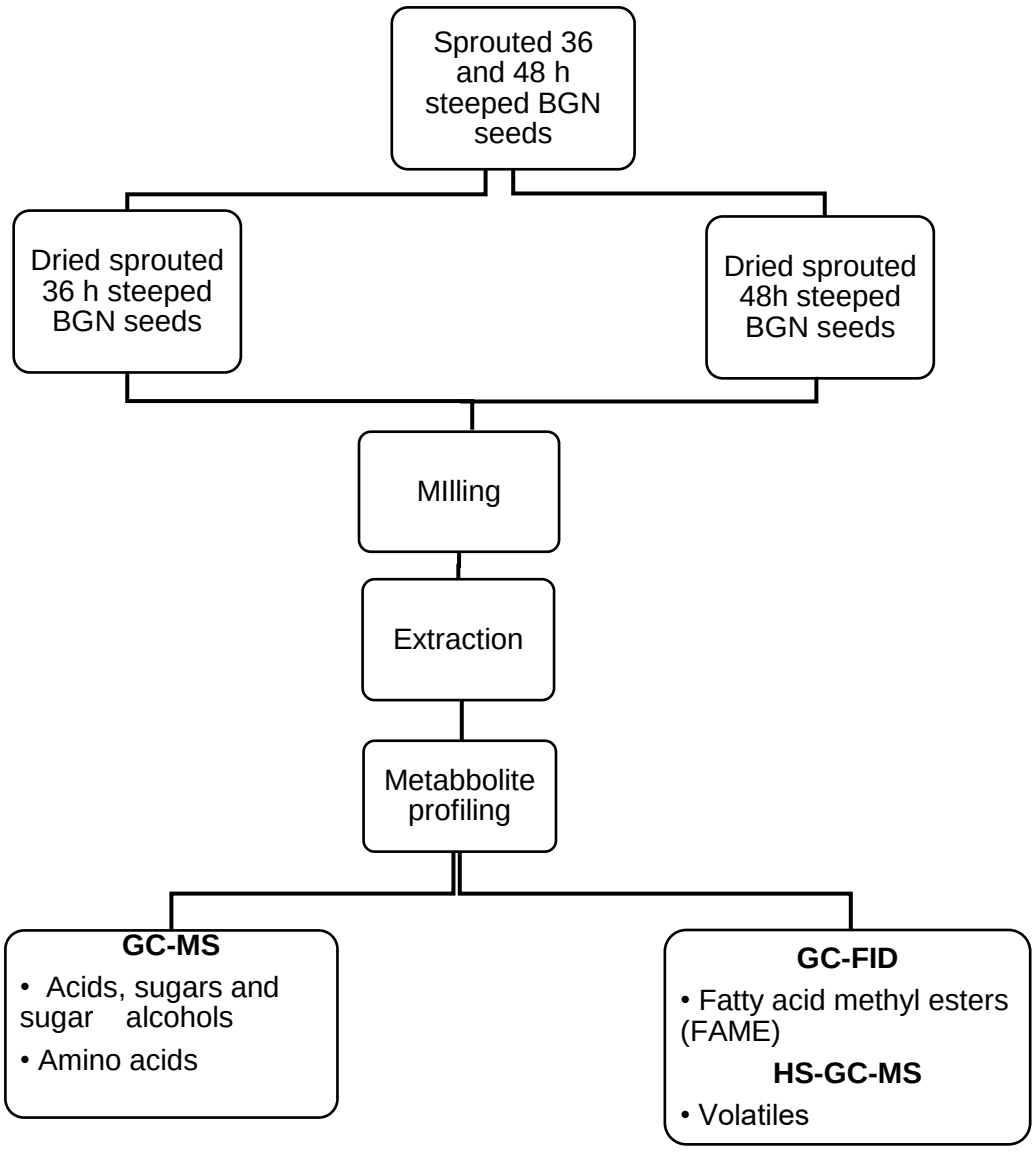


Figure 4.1: Chapter four experimental outline

GC-MS: Gas Chromatography-Mass Spectrometry, GC-FID: Gas Chromatography-Flame Ionization Detection, HS-GC-FID: Headspace Gas Chromatography-Flame Ionization Detector

4.3 Preparation of raw, steeped, and sprouted Bambara Groundnut for Metabolite Profiling

The raw unsprouted BGN seeds and the dried 36 and 48 h steeped BGN seeds sprouted from 0 to 144 h were separately milled using Waring Laboratory Science blender model 7009G (Waring Laboratory Science, CT, USA). The samples were packaged in Ziplock bags and stored at −18°C until analysis. The samples were profiled for fatty acid methyl esters (FAME), sugars, sugar alcohols, organic acids, amino acids, and volatile compounds. Metabolite profiling of sprouted BGN FAME, sugars, sugar alcohols and organic acids were analysed by capillary GC-MS according to the methods of Frank *et al.* (2007, 2009, 2011); Shu *et al.* (2008) and Jom *et al.* (2011). In addition, headspace GC-FID was used to analyse volatile compounds by the method of Salmerón *et al.* (2015).

4.3.1 Fatty Acids Methyl Esters (FAME) and hydrocarbons determination by Gas Chromatography-Flame Ionisation Detector (GC-FID)

The fatty acid and hydrocarbons analysis was carried out by extracting and converting the sample lipids into fatty acid methyl esters (FAME). The extraction was carried out using diethyl ether and petroleum ether in methanol. Using the model Agilent 7890A gas chromatography (GC), the detection was done according to the AOAC (2005) method 996.06 with some modifications.

The samples (un-malted and malted BGN) of 1.5 mg were weighed into the separate 70 mL test tubes to digest. The tube's contents were thoroughly mixed with 100 mg of pyrogallic acid, 2 mL internal standard solution of 5 mg/mL undecanoic acid dissolved in hexane and 2 mL ethanol. Immediately after mixing, 10 mL of 32% HCL was mixed into each tube. The tubes were then placed in a 70–80^{o}C water bath for 40 min and contents mixed every 10 min. After digestion, the tubes were removed and allowed to cool to room temperature. The 25 mL diethyl ether was added to each tube and shook for 5 min for extraction. Petroleum ether of 25 mL was further added and shook for 5 min again. After separating the two layers, the clear upper layer was decanted into 150 mL beakers, and ether was evaporated in the fume hood to dryness.

Derivatisation of the samples was carried out by reconstituting the residues in 3 mL chloroform and diethyl ether. The solutions were transferred into 10 mL tubes and evaporated under the nitrogen stream to dry. Immediately after drying, 2 mL of 2% H_2S0_4 in methanol reagent and 1 mL toluene were added. The tubes were tightly closed and placed in the incubator at 100^{o}C for 45 min. Next, the tubes were cooled to room temperature. After cooling, 5 mL distilled water and 1 mL hexane was added and thoroughly shaken using the vortex mixer for 1 min. The layers were left to separate, and the top layers were carefully transferred to 20 mL test tubes. Approximately 1 g anhydrous Na_2S0_4 was

added to each tube to have a clear solution. The clear solutions were then transferred into 2 mL clear vials, and GC analysis was carried out. Fatty acids were identified by comparing their retention times to the retention times of the standard.

4.3.2 Sugars, acids, and sugar alcohols determination by Gas Chromatography-Mass Spectrometric (GC-MS)

Sugars, acids, and sugar alcohols were analysed using GC-MS by measuring 1 mL of 70% methanol (MeOH) and adding approximately 100 mg of the samples (raw unsprouted and malted BGN) extracting at 45°C in the oven for 3 hours. The extracted samples of 130 µL were dried entirely with a gentle stream of nitrogen and derivatised with 100 µL of methoxamine at 40^{o}C for two h. Then 30 µL of N, O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) was added and derivatised at 60^{o}C for 30 minutes. Finally, the samples were transferred into 2 mL GC vials, and 1 µL was injected onto the GC-MS in splitless mode.

Separation was performed on a gas chromatograph (Trace 1300, Thermo Scientific) coupled to a mass spectrometer (TSQ 8000, Thermo Scientific). Helium was used as the carrier gas at a 1 mL/min flow rate. The carbohydrates were separated on a non-polar Rxi-5Sil MS capillary column (30 m, 0.25 mm ID, 0.25 µm film thickness). The injector temperature was maintained at 250^{o}C. The oven temperature was 80 ^{o}C for 1 min and ramped up to 300^{o}C at a rate of 7^{o}C/min and held for 2 min.

4.3.3 Amino acids determination by Gas Chromatography-Mass Spectrometry (GC-MS)

Following the method of Stenerson (2011), 3 mL of 6 molar mass HCl was added to *approximately* 500 mg of the milled un-malted and malted BGN (36 and 48 h steeped). First, they were hydrolysed for 24 h at 110^{o}C, cooled down to room temperature and diluted at a ratio of 1:9 with 70% methanol (v/v). Next, 100 µL was transferred into a 2 mL tube and dried entirely under a gentle stream of nitrogen. Then, the samples were reconstituted and derivatised with 30 µL silylation reagent N-tert-butyldimethylsilyl- N-methyl trifluoroacetamide (MTBSTFA) and 100 µL acetonitrile at 100^{o}C for 1 h. After which, they were cooled down to room temperature and injected into the GC-MS instrument for analysis.

The components separation was performed on a gas chromatograph (Trace1300, Thermo Scientific) coupled to a TSQ8000 mass spectrometer (Thermo Scientific). The GC-MS system was connected to a TriPLUS auto-sampler. Amino acids were separated on a Rxi-5Sil MS capillary column (30 m, 0.25 mm ID, 0.25 µm film thickness). Helium was used as the carrier gas at a 1 mL/min flow rate. The injector temperature was maintained at 250°C. One microliter of the sample was injected in spitless mode. The oven temperature

was programmed to 100^{o}C for 1 min and ramped up to 300^{o}C at a rate of 15^{o}C/min and held for 6 min. The Agilent mass spectrometer detector (MSD) was operated in scan mode, and the source and quad temperatures were maintained at 250^{o}C and 150^{o}C, respectively. The transfer line temperature was maintained at 250^{o}C. The mass spectrometer was operated under electron impact (EI) mode at ionisation energy of 70eV by scanning from 35 to 650 m/z.

4.3.4 Determination of volatile compounds by Headspace Gas Chromatography-Mass Spectrometry (HS-GC-MS)

The headspace gas chromatography-mass spectrometry (HS–GC–MS) analyses were performed using a model Agilent 7890B Gas Chromatography–5977A coupled with Mass Spectrometer detector system (Santa Clara, CA, USA) with a split-less injector suitable for GC analysis by following the method of (Salmerón *et al.*, 2015) with some differences. The Agilent J&W GC HP-5ms capillary column of 30 m x 0.25 mm x 0.25 µm was used to separate the volatiles. The carrier gas was helium, with a 0.6 mL/min flowrate. Two hundred and fifty microlitres of sample volume were injected (un-malted and malted BGN) with a split ratio of 50:1 and weighed into 10 mL glass headspace vials covered with silicon septum with a purge flow of 3 mL/min and screw-capped. The oven temperature was 50°C held for 5 minutes, increased at 10^{o}C / min to 200°C and held for 5 minutes with a running time of 25 min. The injector temperature, pressures, and volume were set at 240^{o}C, 2.6149 psi and 250 µL, respectively. The incubation temperature and time were set at 120^{o}C and 300 sec, respectively. The samples were then run concurrently.

The compounds were identified through Wiley mass spectral (MS) library and Golm metabolome database search. The volatile compounds identification was by comparing the mass spectra with the spectra of the reference compounds in both the Wiley MS library and verified based on mass spectra obtained from the literature. The volatile results were provided based on the compounds' quality and peak area counts.

4.4 Identification of Metabolite Compounds

Identification of BGN constituents was by comparing retention times and mass spectra with those of reference compounds. Also, by comparing mass spectra with the entries of the National Institute of Standards and Technology mass spectra library NIST02 and the GOLM metabolome database (Shu *et al.*, 2008; Frank *et al.*, 2009; Jom *et al.*, 2011; McGough *et al.*, 2012).

4.5 Statistical Analysis

The results reported were the mean of two independent trials. Multivariate analysis of variance (MANOVA) was used to determine the mean difference between treatments at $p \leq 0.05$. Means were separated by Duncan multiple range tests where differences exist using IBM SPSS version 25 (IBM, 2018). The results were visualised in a heatmap diagram combined with agglomerative categorised clustering of the metabolites identified in the malted BGN seeds.

4.6 Results and Discussion

4.6.1 Fatty Acids Methyl Esters (FAME) of raw, steeped and sprouted Bambara groundnut

The fatty acids were quantified using GC-FID with FAMEs derivatisation, resulting in three FAME metabolites in the raw BGN seeds, as shown in Table 4.1. Mainly, the FAME contents of the unsprouted raw BGN seeds were the linoleic acid methyl ester (C18:2n6c), linolelaidic acid methyl ester (C18:2n6t), and palmitic acid methyl ester (C16:0) with the percentage content in the total lipid of 2.04 , 1.36 and 1.46%, respectively.

Table 4.1 Fatty acid methyl ester (FAME) of raw Bambara groundnut

Compound Name	Retention Time (min)	Peak Area	% FAME mean concentration
Saturated fatty acid (SFA)			
Palmitic acid methyl ester (C16:0)	37.70	1152	1.46 ± 0.05
Polyunsaturated fatty acid (PUFA)			
Linolelaidic acid methyl ester (C18:2n6t)	43.28	1418	1.36 ± 0.06
Linoleic acid methyl ester (C18:2n6c)	44.89	2124	2.04 ± 0.08
Total FAME			4.86 ± 0.19

The abundant fatty acids in the raw BGN seeds were unsaturated, predominantly the polyunsaturated essential fatty acid linoleic acid. Linoleic acids are essential omega-6 fatty acids that the body cannot manufacture and can cause a deficiency in human nutrition if not taken through a food diet (Mori & Hodgson, 2012). In addition, omega-6 fatty acids have been found to reduce serum cholesterol and low-density lipoproteins (LDL) levels in humans when consumed (Mori & Hodgson, 2012; Aremu *et al.*, 2013). Linolelaidic acid methyl ester, an omega-6 trans fatty acid (TFA) also known as 9,12-Octadecadienoic acid,

methyl ester, is an isomer of linoleic acid; which is trans fatty acids are linked to a high risk of cardiovascular diseases (Park, 2009). It, however, declined in this study with the increase in steeping and sprouting time. The decline is in relation to Adebiyi *et al.* (2021) studies on the metabolite profile of Bambara groundnut and *dawadawa* (an African fermented condiment). The linolelaidic acid methyl ester was detected in raw BGN seeds but disappeared in the processed dehulled and un-hulled *dawadawa*. The reduction in linolelaidic showed that the omega-6 trans fatty acid could be reduced in BGN via various processing methods. The saturated FAME detected in raw BGN seeds was palmitic acid methyl ester. Palmitic acid methyl ester, also called hexadecanoic methyl acid, is a long-chain saturated fatty acid commonly found in plants, especially palm oil, animals and humans (Ibrahim *et al.*, 2021). It is an endogenous FAME, which is said to have a potent anti-inflammatory and anti-fibrotic effect on the body (Lin *et al.*, 2019; Wu *et al.*, 2021). In addition, it is known for its essential role as a source of energy for the body and can inhibit cardiac arrest induced by neuroinflammation (Wang *et al.*, 2018; Wu *et al.*, 2021). Also, research on the use of dietary fat supplements rich in palmitic acid on milk yield and composition in the feed of cows and sheep has shown an increase in the performance of their lactation (Daneshvar *et al.*, 2021; Sallam *et al.*, 2021). The palmitic acid methyl ester is used in food production as a food additive and cosmetics as emollient due to its quality ability to soothe the skin (Archambault & Bonté, 2021; Han *et al.*, 2021).

The total saturated fatty acids for the 36 and 48 h steeped BGN seeds were 1.19 and 1.01%, respectively. The total monounsaturated fatty acids were 0.06 and 0% for 36 and 48 h steeped BGN seeds, respectively. While the total polyunsaturated fatty acids were 2.61 and 2.26, respectively, as shown in Table 4.2. Based on the multivariant test, there was no significant difference in the 36 and 48 h steeped BGN seeds but the concentration of the FAME reduced with an increase in steeping time.

Table 4.2 Fatty Acid Methyl Acid concentration in steeped Bambara groundnut[1]

	Steeping (h)	
FAME concentration %	**36**	**48**
Saturated Fat	1.19 ± 0.31[a]	1.01 ± 0.25[a]
Monounsaturated	0.06 ± 0.12[a]	0.00 ± 0.00[a]
Polyunsaturated	2.61 ± 0.84[a]	2.26 ± 0.64[a]
Total FAME	3.86 ± 1.27[a]	3.27 ± 0.89[a]

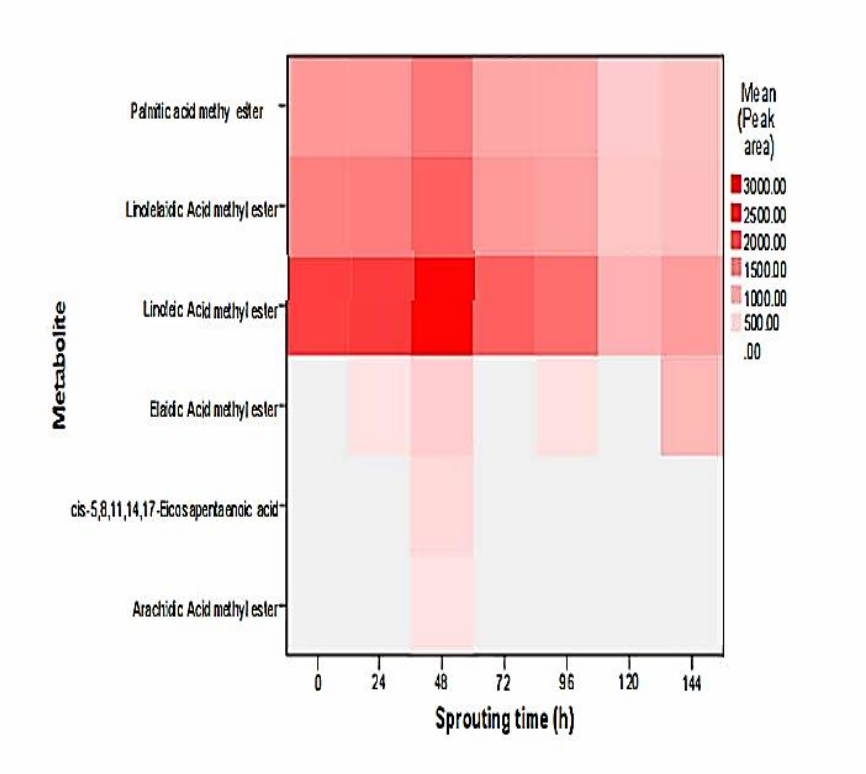
[1]Values are mean of duplicates ± standard deviation, mean values with the same superscripts within the same row are not significantly different. FAME: Fatty Acid Methyl Acid

The total FAME concentration for the 36 and 48 h steeped BGN seeds were 3.86 and 3.27%, respectively. The 36 and 48 h steeping of BGN significantly decreased contents of FAME compared to the raw BGN (4.86%) seeds.

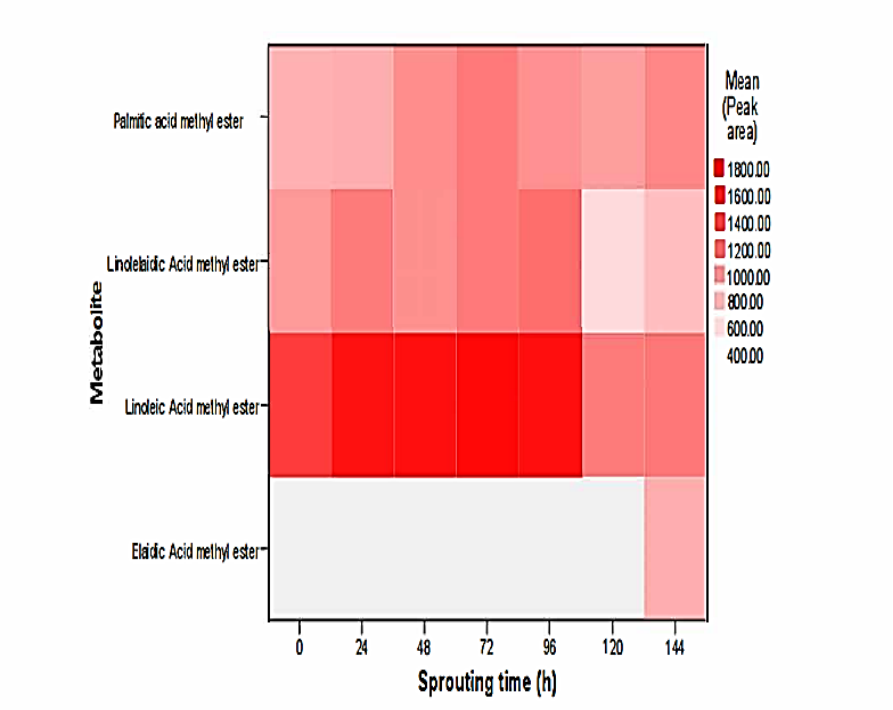
Figure 4.2 shows the detected FAME concentration of sprouted 36 and 48 h steeped BGN seeds as affected by sprouting time. The metabolite levels on the heatmap correspond to the colour temperature, and higher temperatures indicated higher levels of FAME compounds. Six and four FAME metabolites were detected for the sprouted 36 h and 48 h steeped BGN seeds, respectively. However, steeping BGN seeds up to 48 h resulted in the loss of cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3) and arachidic acid methyl ester (C20:0). The linoleic acid methyl ester (C18:2n6c), palmitic acid methyl ester (C16:0) and linolelaidic acid methyl ester (C18:2n6t) were the most dominant FAME in the sprouted seeds for the two steeping regimes. The dominant FAMEs were shown to be at their highest concentration from 24 to 96 h of sprouting for the two steeping times, then a decline. The dominant FAME was highest in the 36 h steeping; thus, steeping for 36 h and sprouting for 96 h could give an abundance of the linoleic acid methyl ester and palmitic acid methyl ester to access their anti-inflammatory and antioxidant components (Kolar *et al.*, 2019; Salisu *et al.*, 2019).

Elaidic acid methyl ester (C18:1n9t) was dominant at the 144 h sprouting time for both the steeping regimes, as shown in Figure 4.2. Elaidic acid is an isomer of oleic acid, an unsaturated, trans fatty acid, with code C18:1 trans-9 (Ahmed & El-Sisy, 2021; Wang *et al.*, 2021b). It is a major trans-fat found in hydrogenated vegetable oils and margarine, which has been attributed to many diseases, especially heart-related diseases (Vendel Nielsen *et al.*, 2013; Iino *et al.*, 2021). However, it is in low quantities in the two steeping regimes, which correlates with Megat Rusydi *et al.* (2011), who noted its low concentration as sprouting progresses in the kidney beans, mung and peanut. However, the lower concentration of elaidic acid methyl ester (C18:1n9t) is attributed to the higher lipase activity during germination (Guo *et al.*, 2021). Therefore, since sprouting up to 144 h would make elaidic acid methyl esters abundant, 96 h sprouting could be the optimum sprouting time to avoid the abundance of this fatty acid.

Cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3) and arachidic acid methyl ester (C20:0) were detected only in the 36 h steeped seeds at 48 h sprouting as shown in Figure 4.2. Cis-5,8,11,14,17-Eicosapentaenoic acid is a polyunsaturated longer-chain fatty acid.



(a)



(b)

Figure 4.2: Fatty acid methyl ester (FAME) heat map for (a) 36 h (b) 48 h steeped Bambara groundnut as affected by sprouting time

It plays a vital function in biosynthetic precursors, as cellular membrane components, and protects against oxidative stress (Shi, 2006). Eicosapentaenoic acid is an omega-3 fatty acid essential for human physiology and health. Although it is mainly found in fish, it has been synthesised from plant foods (Paquin, 2009; Gu *et al.*, 2021; Marques *et al.*, 2021). It is known for reducing heart disease risk and anti-inflammatory effects (Bagchi *et al.*, 2015; Marques *et al.*, 2021; Watanabe & Tatsuno, 2021). Plant foods such as walnut, flaxseed, chia seed, soybean, and canola oils, contain alpha-linolenic acid synthesised to eicosapentaenoic acid in the human body at a slow and insufficient rate (Gruenwald, 2009; Grumezescu & Maria Holban, 2019; Seçkin *et al.*, 2021). Hence steeping for 36 h and sprouting for 48 h is recommended for BGN to maximise eicosanoic acid production.

The arachidic acid methyl ester, also known as eicosanoic acid, is a 20-carbon chain fatty acid with cis double bonds belonging to the omega-6 (n-6) polyunsaturated fatty acids (Tallima & El Ridi, 2018; Oppedisano *et al.*, 2020). It is a derivative of essential fatty acids, linolenic acid, and linoleic acid (Alhassanm *et al.*, 2014). Arachidic acid is mostly found in peanuts as well as in BGN seeds, indicating BGN oil is rich in essential fatty acids that can be beneficial to health (Alhassanm *et al.*, 2014; Feldman *et al.*, 2019). They are necessary for synthesising prostaglandins, thromboxane, and leukotrienes hormones (Omotayo & Aremu, 2021). Arachidic acid is a vital ingredient in the pharmaceutical, cosmetics, and food packaging industries (Máthé, 2015). Arachidic acid though low in the 36 h steeped BGN seeds and not present in the 48 h steeped seeds, sprouting for 48 h could allow production of arachidic rich product.

All the detected compounds were reduced with increasing sprouting time for the steeping regime. Such reductions in FAME have also been noticed during the germination of rice seeds, barley, soybeans, chickpea, mung beans (Shu *et al.*, 2008; Megat Rusydi *et al.*, 2011; Vasishtha & Srivastava, 2012; Nelson *et al.*, 2013; Özcan *et al.*, 2018). The decrease was, however, attributed to the lipid enzymatic degradation. However, Frank *et al.* (2011) noted that the rate of lipid degradation is mainly influenced by the malting process (temperature, soaking, and germination time). Furthermore, the reduction could also be due to the important lipases associated with the lipid catalysis during the germination of the seeds (Jom *et al.*, 2011; Nelson *et al.*, 2013; Winarsi *et al.*, 2021). The lipases catalyse the hydrolysis of ester carboxylate bonds by liberating organic alcohols and fatty acids (Joshi, 2018; Guo *et al.*, 2021; Winarsi *et al.*, 2021). The FAMEs are more abundant in the 48 h steeped BGN seeds; however, there were losses to some FAMEs that were detected in the 36 h steeped seeds. Thus, steeping for 36 h and sprouting up to 96 hours could produce steeped BGN seeds with essential FAMEs that could benefit the healthy living.

4.6.2 Acids, sugar, and sugar alcohol of raw, steeped, and sprouted BGN

The mean lactic acid was 0.01 mg/g for raw BGN seeds, 0.01 mg/g for 36 h steeped BGN seeds and 0.01 mg/g for 48 h steeped BGN seeds. Fructose concentration was 0.04, 0.17, , and 0.01 mg/g for BGN raw seeds, 36 and 48 h steeped seeds, respectively. Myo-inositol was 0.22, 0.22 and 0.24 mg/g for BGN raw seeds, 36 and 48 h steeped seeds. Sucrose was 3.96, 6.62 and 6.23 mg/g for BGN raw seeds, 36 and 48 h steeped seeds, as illustrated in Figure 4.3. There was a significant ($p = 0.000$) difference for the acids, sugar, and sugar alcohol based on steeping time. There was a decrease in the lactic acid, fructose and sucrose concentration as steeping time increased except for myo-inositol, the sugar alcohol, which increased. The result of this study agrees with the increase in lactic acid content for soybeans during the first six hours of soaking at 30°C, after which it decreased (Mulyowidarso *et al.*, 2007).

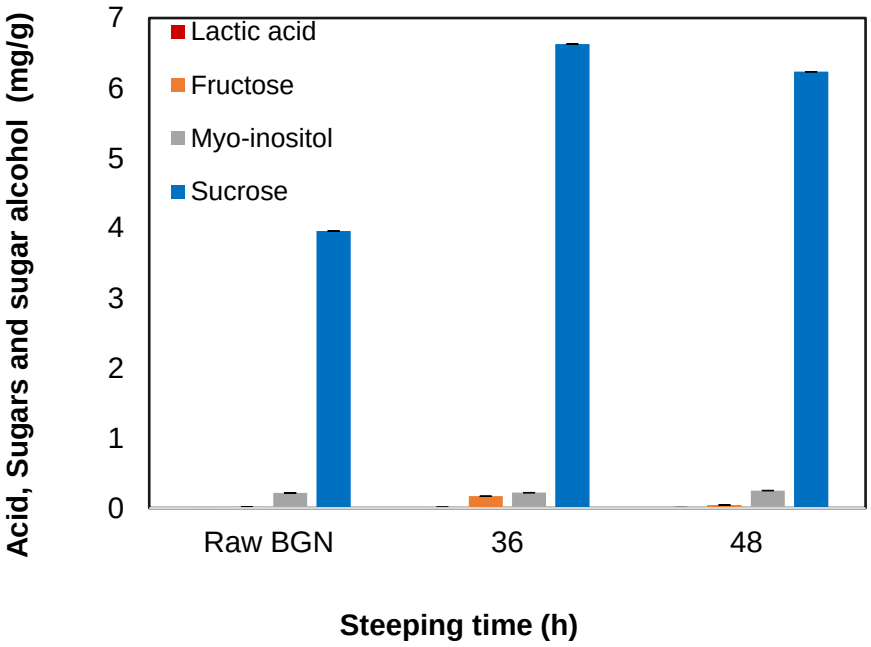


Figure 4.3: Impact of steeping on the Bambara groundnut sugars, organic acids, and sugar alcohols content

Fructose and sucrose contents reduction agrees with Mubaiwa *et al.* (2018), where BGN was soaked to ascertain the functional properties of red and black-eyed seeded BGN

seeds. El-safy *et al.* (2013) also noted a reduction of sugar during the soaking of some legumes and cereals grains. However, the reduction during soaking was attributed to the diffusion of sugars into the soaking water after solubilisation. It has also been noted that soaking reduces the level of sugars in pulses, seeds and cereal (Jood *et al.*, 1988; Hooda and Jood, 2003; Mubaiwa *et al.*, 2018; Liu *et al.*, 2020). In contrast to this sugar reduction trend, Vidal-Valverde *et al.* (1992) and Omoikhoje *et al.* (2006) noted an increase in fructose and sucrose in lentils and BGN after soaking.

Based on sprouting time, the lactic acid, fructose, myo-inositol, and sucrose were significantly ($p = 0.000$) different across the sprouting times for the two steeping regimes, as shown in Table 4.3. Lactic acid decreased for the 36 and 48 h steeped BGN seeds during sprouting. The lactic acid concentration for 36 h steeped BGN ranged from 0 to 0.05 while 48 h steeped BGN concentration ranged from 0 to 0.02. The concentration of fructose was in the range of 0 to 0.8 for 36 h steeped BGN seeds and 0.01 to 0.15 for 48 steeped BGN seeds.

Table 4.3 Acid, Sugar alcohol and sugar content of sprouted Bambara groundnut[1]

		36 h steeping (h)		
Sprouting time (h)	**Lactic acid (mg/g)**	**Myoinositol (mg/g)**	**Fructose (mg/g)**	**Sucrose (mg/g)**
0	0.05 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.01^{a}	5.39 ± 0.01^{a}
24	0.00 ± 0.00^{b}	0.26 ± 0.00^{b}	0.02 ± 0.01^{a}	5.15 ± 0.01^{b}
48	0.00 ± 0.00c	0.18 ± 0.00^{c}	0.00 ± 0.00^{b}	6.02 ± 0.01^{c}
72	0.05 ± 0.01^{b}	0.19 ± 0.00^{c}	0.00 ± 0.00^{b}	9.95 ± 0.02^{d}
96	0.01 ± 0.01^{b}	0.22 ± 0.00^{d}	0.02 ± 0.00^{ac}	7.16 ± 0.01^{e}
120	0.00 ± 0.01^{b}	0.35 ± 0.01^{e}	0.29 ± 0.00^{d}	6.85 ± 0.02^{f}
144	0.01 ± 0.00^{d}	0.34 ± 0.00^{f}	0.83 ± 0.00^{e}	5.90 ± 0.00^{g}
		48 h steeping (h)		
0	0.01 ± 0.00^{a}	0.09 ± 0.01^{a}	0.04 ± 0.01^{a}	3.53 ± 0.00^{a}
24	0.00 ± 0.00^{b}	0.33 ± 0.00^{b}	0.02 ± 0.00^{b}	8.92 ± 0.00^{be}
48	0.00 ± 0.01^{b}	0.33 ± 0.00^{b}	0.04 ± 0.01^{c}	9.67 ± 0.00^{a}
72	0.01 ± 0.00^{c}	0.26 ± 0.00^{c}	0.15 ± 0.00^{d}	6.01 ± 0.01^{c}
96	0.01 ± 0.00^{b}	0.25 ± 0.01^{d}	0.01 ± 0.00^{e}	4.79 ± 0.01^{d}
120	0.01 ± 0.00^{c}	0.29 ± 0.01^{e}	0.02 ± 0.01^{b}	5.88 ± 0.01^{e}
144	0.00 ± 0.01^{b}	0.22 ± 0.00^{f}	0.02 ± 0.00^{be}	4.84 ± 0.01^{bd}

[1]Values are mean of duplicates ± standard deviation, mean values with different superscripts within the same column are significantly ($p \leq 0.05$) different

Myo-inositol ranged from 0.01 to 0.34 mg/g and 0.09 to 0.33 mg/g for both steeping regimes. Sucrose ranged from 5.39 to 9.95 mg/g and 3.53 to 9.67 mg/g for the 36 and 48 h steeped BGN seeds, respectively. The lactic acid concentration increased during steeping and decreased as germination continued for both the steeping regimes. This same trend was observed during barley malting by Xiang *et al.* (2006), where lactic acid content increased during steeping and decreased with increased germination time. However, contrary to the result of Xiang *et al.*(2006), Shu *et al.* (2008), Frank *et al.* (2011) and Jom *et al.* (2011) observed an increase in lactic acid during the germination of barley, mung beans, and rice. Fructose increased up to 24 h, not detected in 48 and 72 h and then increased until the end of sprouting for 36 h steeping. Observed during buckwheat, soybean and rice germination by Zhao *et al.* (2021), Gu *et al.*(2017), and Shu *et al.* (2008) is the trend in this study for 36 h steeped BGN seeds where the levels of fructose increased at the beginning of germination then decreased with continuous germination. After that, the fructose and other sugars significantly increased until the end of germination. The increasing trends was attributed to the proteolytical enzyme contributing factors activities in grains. However, the trend in 48 h steeping showed an increase up to 72 h sprouting and then decline up to the end of sprouting time. The decreasing fructose of the 48 h steeped BGN seeds is similar to germinating barley, mung beans, peas and lentil seeds (El-Adawy *et al.*, 2003; Frank *et al.*, 2011; Jom *et al.*, 2011; Gorzolka *et al.*, 2012). The decrease in fructose was reported due to their utilisation as an energy source to start germination (El-Adawy *et al.*, 2003; Mubarak, 2005; Frank *et al.*, 2011).

The sucrose concentration increased at the beginning of sprouting but reduced at the end of the sprouting period for both 36 and 48 h steeped BGN seeds. High sucrose concentrations were observed at the 72 and 48 h sprouting times for the 36 and 48 h steeping regimes, respectively. A similar trend was observed for sprouted mung bean where the concentration of sucrose increased within the first 24 h and reduced afterwards (Jom *et al.*, 2011). Also, the sucrose concentration decreased as sprouting increased for soybean sprouts during germination. The increase in sucrose was proposed to be due to the metabolomics pathway linked with energy production (Gu *et al.*, 2017). The reduction in sucrose was also attributed to a lack of raffinose due to the hydrolysis of sucrose to supply energy towards the end of sprouting time (Mubarak, 2005; Tang *et al.*, 2014b).

The myo-inositol did not have a consistent increase pattern during sprouting for both steeping regimes but was high in concentration in the 120 and 48 h sprouting time for the 36 and 48 h steeping, respectively. The inconsistent increase in myo-inositol was also observed in germinating rice by Shu *et al.* (2008). Phytases are activated during the germination of cereal and legumes to make available secretion of phosphate, mineral elements, and myo-inositol for plant growth and development (Salem *et al.*, 2014; Lemmens

et al., 2019). The secretion of myo-inositol by phytate during germination can be attributed to the concentration of myo-inositol in BGN seeds and the reduction of phytic acid (Limwiwattana *et al.*, 2016; Erba *et al.*, 2019). In recent studies in comparison to this study, elevated myo-inositol towards the end of sprouting was detected during sprouting of barley, soybean, black gram beans and mung bean (Frank *et al.*, 2011; Jom *et al.*, 2011; Limwiwattana *et al.*, 2016; Gu *et al.*, 2017). Myo-inositol is a C6 sugar alcohol that can be used to regulate thyroid- and follicle-stimulating hormones and insulin (Croze and Soulage, 2013; Unfer *et al.*, 2017). Therefore, its higher quantity in the sprouted BGN could encourage steeping at 36 h and sprouting up to 144 h to optimise its production.

Today's consumers are conscious of what is put in the production of foods and drinks, which has encouraged the use of malt sugars in the food industries as a healthy version of granulated sugar (Chaves *et al.*, 2019; Targan, 2020). Also, malt sugars in the form of malt syrups have been studied and confirmed to have various antioxidant components that could benefit consumers' health (Ahmed, 2022). Therefore, to optimize the sugars in the BGN malt, BGN could be steeped at 36 h and sprouted for 72 h.

4.6.3 The amino acid content of raw, steeped and sprouted Bambara groundnut

There were seven essential amino acids (EAA) (lysine, methionine, leucine, isoleucine, valine, threonine, and phenylalanine) and eight non-essential amino acids (NEAA) (serine, glycine, alanine, tyrosine, proline, aspartic acid, cysteine, and glutamic acid) detected in the raw BGN seeds. The total essential amino acids and non-essential amino acids were 108.67and 80.81 mg/g for the raw BGN seeds, as shown in Table 4.4. Lysine (49.49 mg/g) was the highest in the essential amino acids, while glutamic and aspartic acids were the highest in the non-essential amino acids. The amino acid concentrations in the raw BGN seeds agreed with previous studies, where the lysine, aspartic, and glutamic acids (18.28 and 17.20 mg/g) were of higher concentrations than the other detected amino acids (Abdualrahman *et al.*, 2015; Yao *et al.*, 2015). Methionine, the sulphur-containing amino acid, was the lowest. Bambara groundnut seeds, like other legumes, are low in methionine and pairing it with high sulphur-containing cereals like maize has been encouraged (Mubaiwa *et al.*, 2018; Acquah *et al.*, 2021). The composition of the raw BGN seed amino acid profile has further established that BGN seeds are a good source of essential and non-essential amino acids that could help combat protein deficiencies in our diet by combining them with other food products.

Table 4.4 Amino Acids concentration of raw Bambara groundnut[1]

Amino Acids	Amino acids Concentration (mg/g)
Essential	
Lysine	49.94 ±0.36
Methionine	3.30 ± 0.03
Leucine	12.84 ± 0.00
Isoleucine	8.24 ± 0.08
Valine	10.39 ± 0.12
Threonine	12.48 ± 0.09
Phenylalanine	11.48 ± 0.06
Total EAA	**108.67 ± 0.74**
Non-essential	
Serine	9.43 ± 0.01
Glycine	5.55 ± 0.06
Alanine	6.46 ± 0.04
Tyrosine	3.90 ± 0.04
Proline	10.24 ± 0.00
Aspartic acid	18.28 ± 0.09
Cysteine	9.75 ± 0.18
Glutamic acid	17.20 ± 0.05
Total NEAA	**80.81 ± 0.47**

[1]Values are mean of duplicates ± standard deviation of duplicate readings. EAA- essential amino acid, NEAA- non-essential amino acid

The total essential and non-essential amino acids for the 36 and 48 h steeped BGN seeds were 65.12 and 195.10 mg/g, respectively, as shown in Table 4.5. The concentration of the amino acids based on steeping time significantly ($p = 0.000$) differed. Hence, the differences in steeping time (36 and 48 h) affected the amino acid concentration. The increase in the amino acid concentration agrees with studies on the amino acid of steeped BGN seeds (Nzelu, 2016). The higher amino acid has been attributed to increased exposure to hydration as more water is imbibed in the 48 h steeping, causing an increase in modification due to increases in metabolic activity (Turner *et al.*, 2019). The aspartic acid, glutamic acid and arginine are high, while the glutamic and aspartic acid were the most abundant amino acids in the raw, 36 and 48 h steeped BGN seeds as recorded in BGN and other legumes (Yao *et al.*, 2015; Azman *et al.*, 2019).

Table 4.5 Amino Acid profile of 36 and 48 h steeped Bambara groundnut[1]

	Steeping time (h)	
Amino Acids (mg/g)	**36**	**48**
Essential		
Lysine	25.51 ± 13.43[a]	100.32 ± 33.37[b]
Methionine	1.69 ± 0.32[a]	4.67 ± 0.83[b]
Leucine	7.25 ± 1.57[a]	20.00 ± 3.33[b]
Isoleucine	6.28 ± 1.37[a]	15.18 ± 2.25[b]
Valine	7.52 ± 1.29[a]	17.97 ± 2.78[b]
Threonine	9.66 ± 1.65[a]	21.45 ± 5.40[a]
Phenylalanine	7.22 ± 1.06[a]	15.51 ± 2.14[b]
Total EAA	**65.12 ± 2.70[a]**	**195.10 ± 50.08[b]**
Non-essential		
Serine	7.23 ± 1.05[a]	19.13± 3.72[b]
Glycine	3.55 ± 0.82[a]	7.16 ± 0.66[b]
Alanine	4.11 ± 1.04[a]	8.26 ±0.73[b]
Tyrosine	2.46 ± 0.85[a]	4.66 ± 0.39[b]
Proline	6.64 ± 1.86[a]	12.44 ± 3.37[b]
Aspartic acid	11.4 ± 2.47[a]	19.23 ± 1.07[b]
Cysteine	4.64 ± 2.06[a]	5.78 ± 4.67[b]
Glutamic acid	11.61 ± 2.94[a]	14.02 ± 3.22[b]
Total NEAA	**51.64 ± 13.10[a]**	**90.68 ± 17.81[b]**

[1]Values are mean of duplicates ± standard deviation, mean values with different superscripts within the same row are significantly different. EAA-Essential amino acid, NEAA-non-essential amino acid

There was a significant ($p = 0.000$) difference in the amino acid concentration from 0 to 144 h sprouting time. The essential amino acids (lysine, threonine, valine, phenylalanine, leucine, isoleucine, and methionine) were reduced with an increase in sprouting time for the two steeping regimes shown in Tables 4.6 and 4.7. Lysine was the most abundant amino acid of all the essential amino acid concentrations.

Table 4.6 Effect of sprouting time on the amino acid contents of 36 h steeped Bambara groundnut[1]

Amino acids (mg/g)	Sprouting time (h)						
Essential	**0**	**24**	**48**	**72**	**96**	**120**	**144**
Lysine	36.75 ± 0.42^{a}	31.39 ± 1.05^{b}	44.89 ± 0.25^{c}	26.11 ± 0.06^{d}	23.98 ± 0.44^{e}	7.76 ± 0.23^{f}	7.69 ± 0.29^{f}
Threonine	13.39 ± 0.11^{a}	8.02 ± 0.21^{b}	9.40 ± 0.05^{c}	9.24 ± 0.00^{cd}	9.04 ± 0.02^{df}	9.65 ± 0.06^{e}	8.93 ± 0.10^{f}
Valine	9.21 ± 0.28^{a}	7.28 ± 0.12^{b}	8.69 ± 0.04^{c}	8.38 ± 0.13^{c}	7.35 ± 0.26^{d}	6.14 ± 0.05^{e}	5.59 ± 0.13^{f}
Phenylalanine	9.13 ± 0.36^{a}	6.87 ± 0.13^{be}	8.26 ± 0.04^{c}	6.35 ± 0.21^{df}	7.22 ± 0.13^{e}	6.12 ± 0.05^{d}	6.59 ± 0.06^{bf}
Leucine	8.90 ± 0.17^{a}	7.04 ± 0.04^{b}	9.30 ± 0.00^{c}	6.67 ± 0.35^{d}	8.26 ± 0.00^{e}	5.43 ± 0.02^{f}	5.18 ± 0.06^{f}
Isoleucine	8.70 ± 0.25^{a}	6.71 ± 0.14^{b}	6.80 ± 0.01^{b}	6.67 ± 0.12^{b}	5.80 ± 0.16^{c}	4.70 ± 0.00^{d}	4.57 ± 0.01^{d}
Methionine	2.10 ± 0.03^{a}	1.24 ± 0.28^{b}	1.64 ± 0.01^{c}	1.87 ± 0.01^{d}	2.06 ± 0.035^{a}	1.38 ± 0.04^{e}	1.54 ± 0.02^{f}
Non-essential							
Aspartic acid	15.80 ± 0.47^{a}	11.13 ± 0.26^{b}	12.88 ± 0.06^{c}	12.27 ± 0.06^{d}	10.7 ± 0.02^{b}	8.81 ± 0.11^{e}	8.23 ± 0.01^{f}
Glutamic acid	15.47 ± 0.15^{a}	15.17 ± 0.01^{a}	12.03 ± 0.13^{b}	12.5 ± 0.37^{c}	9.9 ± 0.06^{d}	7.81 ± 0.01^{e}	8.41 ± 0.05^{f}
Proline	10.76 ± 0.25^{a}	5.78 ± 0.20^{b}	6.96 ± 0.22^{c}	6.35 ± 0.10^{d}	6.27 ± 0.11^{d}	4.95 ± 0.11^{e}	5.42 ± 0.24^{b}
Serine	9.01 ± 0.13^{a}	5.94 ± 0.08^{b}	6.80 ± 0.04^{c}	8.14 ± 0.04^{d}	6.22 ± 0.06^{e}	6.91 ± 0.08^{c}	7.62 ± 0.07^{f}
Cysteine	6.15 ± 0.25^{ab}	6.92 ± 0.95^{a}	5.30 ± 0.01^{b}	6.79 ± 0.51^{a}	2.16 ± 0.21^{c}	2.43 ± 0.04^{c}	2.76 ± 0.08^{c}
Alanine	6.14 ± 0.02a	3.46 ± 0.03^{b}	4.68 ± 0.02^{c}	4.39 ± 0.06^{d}	3.87 ± 0.02^{e}	2.98 ± 0.01^{f}	3.25 ± 0.04^{g}
Glycine	5.16 ± 0.05^{a}	3.72 ± 0.01^{b}	3.88 ± 0.01^{c}	3.02 ± 0.02^{d}	2.78 ± 0.04^{e}	2.67 ± 0.03^{f}	3.64 ± 0.06^{b}
Tyrosine	2.84 ± 0.08^{a}	2.86 ± 0.04^{a}	3.67 ± 0.01^{b}	3.13 ± 0.02^{a}	1.90 ± 0.04^{c}	1.44 ± 0.01^{d}	1.41 ± 0.33^{d}

[1]Values are the mean of duplicates ± standard deviation of amino acid concentrations for the 36 h steeped BGN seeds from 0 to 144 h sprouting. Mean values with different superscripts within the same row are significantly different at p ≤ 0 05.

Table 4.7 Effect of sprouting time on the amino acid contents of 48 h steeped Bambara groundnut[1]

Amino acids (mg/g)	Sprouting time (h)						
Essential	**0**	**24**	**48**	**72**	**96**	**120**	**144**
Lysine	41.69 ± 0.49^{a}	137.41 ± 0.49^{b}	131.6 ± 2.27^{b}	103.93 ± 8.51^{c}	68.7 ± 4.82^{d}	119.57 ± 0.59^{e}	99.37 ± 4.67^{c}
Threonine	14.43 ± 0.01^{a}	30.65 ± 0.26^{b}	19.96 ± 0.30^{c}	23.26 ± 0.01^{d}	16.13 ± 0.06^{e}	25.71 ± 0.01^{f}	20.01 ± 1.66^{c}
Valine	12.8 ± 0.06^{a}	20.08 ± 0.46^{bc}	19.81 ± 0.01^{bc}	16.75 ± 0.93^{d}	18.52 ± 0.50^{cd}	21.15 ± 0.45^{b}	16.71 ± 1.60^{d}
Phenylalanine	12.37 ± 0.16^{a}	18.82 ± 0.00^{b}	17.38 ± 0.09^{c}	15.08 ± 0.45^{d}	14.61 ± 0.17^{d}	16.57 ± 0.30^{e}	13.78 ± 0.15^{f}
Leucine	14.39 ± 0.39^{a}	18.18 ± 0.62^{b}	18.89 ± 0.18^{bc}	21.40 ± 0.82^{cd}	21.87 ± 0.52^{d}	25.13 ± 0.71^{e}	20.12 ± 2.45^{bcd}
Isoleucine	10.44 ± 0.11^{a}	16.67 ± 0.12^{b}	16.13 ± 0.08^{c}	17.30 ± 0.31^{d}	14.90 ± 0.40^{e}	16.51 ± 0.00^{cb}	14.31 ± 0.00^{f}
Methionine	3.71 ± 0.05^{a}	5.86 ± 0.04^{b}	5.16 ± 0.04^{c}	3.80 ± 0.06^{a}	4.07 ± 0.04^{d}	5.54 ± 0.00^{e}	4.58 ± 0.57^{f}
Non-essential							
Aspartic acid	18.45 ± 0.16^{a}	19.46 ± 0.03^{b}	19.63 ± 0.16^{b}	17.67 ± 0.05^{c}	18.72 ± 0.19^{a}	21.14 ± 0.04^{d}	19.56 ± 0.37^{b}
Glutamic acid	20.67 ± 0.81^{a}	14.06 ± 0.08^{b}	13.99 ± 0.06^{b}	10.49 ± 0.06^{c}	11.55 ± 0.11^{d}	15.13 ± 0.02e	12.29 ± 0.07^{f}
Proline	6.37 ± 7.26^{a}	13.55 ± 0.17^{b}	13.53 ± 0.01^{b}	13.64 ± 0.16^{b}	11.97 ± 0.18^{ab}	14.79 ± 0.08^{b}	13.26 ± 0.08^{b}
Serine	11.40 ± 0.02^{a}	21.56 ± 0.08^{b}	21.55 ± 0.05^{b}	17.36 ± 0.15^{c}	19.68 ± 0.06^{d}	22.94 ± 0.08^{e}	19.41 ± 0.48^{d}
Cysteine	16.00 ± 0.83^{a}	6.80 ± 0.01^{b}	3.12 ± 0.01^{c}	2.66 ± 0.04^{c}	1.82 ± 0.04^{d}	6.10 ± 0.12^{b}	4.00 ± 0.07^{e}
Alanine	7.74 ± 0.04^{a}	8.41 ± 0.00^{b}	8.75 ± 0.00^{c}	9.13 ± 0.01^{d}	6.99 ± 0.01^{e}	8.89 ± 0.00^{f}	7.91 ± 0.03^{g}
Glycine	6.16 ± 0.01^{a}	7.20 ± 0.04^{b}	7.61 ± 0.04^{c}	8.08 ± 0.04^{d}	6.57 ± 0.05^{e}	7.69 ± 0.03^{c}	6.80 ± 0.15^{f}
Tyrosine	4.69 ± 0.01^{ab}	4.71 ± 0.01^{b}	4.76 ± 0.04^{b}	4.16 ± 0.04^{c}	4.28 ± 0.04^{d}	5.43 ± 0.01^{e}	4.60 ± 0.08^{a}

[1]Values are the mean of duplicates ± standard deviation of amino acid concentrations for the 48 h steeped BGN seeds from 0 to 144 h sprouting. Mean values with different superscripts within the same row are significantly different at p ≤ 0 05.

However, lysine was at its highest concentrations at the 48 h sprouting with a mean concentration of 44.89 mg/g for the 36 h steeping. Meanwhile, the 48 h steeped BGN seeds lysine highest mean concentration of 137.41 mg/g was on the 24 h sprouting time. Also, methionine, sulphur-containing amino acid, improved with increased sprouting time and was highest at the 24 h sprouting for the 48 h steeped BGN seeds. The improved essential amino acids of sprouted BGN in this study agrees with the study of Awobusuyi & Siwela (2019). They noted that *amahewu*, a fermented non-alcoholic cereal grain beverage composited with germinated BGN flour, had the highest essential amino acids, including lysine.

Essential amino acids are categorised as those amino acids whose carbon skeleton cannot be synthesised by the body or can only be synthesised in insufficient amounts and must be supplied through the diet to meet optimal nutritional needs (Wu, 2009; Mota *et al.*, 2016). For example, lysine, which is important for the growth and maintenance of the body, is an abundant essential amino acid in the 36 and 48 h steeped seeds but was at their highest at 48 h steeped and 24 h sprouted BGN seeds. Generally, most legumes are rich in lysine and can be enhanced or improved by sprouting, as shown in this study (Bhat & Karim, 2009; Martín-Cabrejas, 2019).

The non-essential amino acids consisting of aspartic acid, glutamic acid, proline, serine, cysteine, alanine, glycine, and tyrosine did not progressively increase with sprouting time. They were, however, significantly ($p= 0.000$) different throughout the sprouting time. Aspartic and glutamic acid had the highest concentrations for the two steeping regimes throughout the sprouting period. However, 48 h steeped seeds exhibited higher concentrations from 0 to 144 h sprouting for the two abundant non-essential amino acids. Aspartic acids ranged from 8.23 to 15.80 mg/g for 36 h steeping, while 48 h steeping was from 17.67 to 21.14 mg/g. Glutamic acid ranged from 9.9 to 15.47 mg/g and 10.49 to 20.67 mg/g for the two steeping regimes, respectively. The change in amino acid concentration agrees with Nzelu (2016), who reported enhancement of the essential amino acids in malted BGN. However, the change in amino acid was also attributed to the degradation of storage protein, synthesis of new protein, and other materials (Nzelu, 2016). Also, increased concentration of amino acids in germinated mung beans and soybeans have been observed (Mubarak, 2005; Frank *et al.*, 2009; Jom *et al.*, 2011). The increase was attributed to the proteolytic cleavage of the proteins during germination (Shu *et al.*, 2008; Frank *et al.*, 2011).

Studies have shown that the protein content of legumes, BGN inclusive, increase during germination due to the biochemical changes prompted by sprouting by the production of free linking amino acids (Ijarotimi & Esho, 2009; Onwuka & Ezemba, 2013).

However, this study showed inconsistent mean amino acid concentrations as sprouting time increased for the two steeping regimes. Nzelu (2016) noted the same trend and attributed the change in the amino acid content of sprouted BGN to the soaking and germination processes that encouraged modifications whereby some may experience an increase or decrease in quantities. This study has further proven that BGN is a good source of essential amino acids, and they could be enhanced by malting (Ijarotimi & Esho, 2009; Nzelu, 2016; Duodu & Apea-Bah, 2017; Temegne *et al.*, 2018; Muhammad *et al.*, 2020). Steeping for 48 h and 48 h sprouting could also provide a product rich in essential and non-essential amino acids.

4.6.4 Volatile compounds in raw, steeped and sprouted Bambara groundnut

Ten volatiles were identified in the raw BGN seeds, consisting of hydrocarbons, organic acids, alkanes, ketones, and aromatics compounds. The changes in all the volatile compounds were illustrated using a heatmap in Figure 4.4. The metabolite levels on the heatmap correspond to the colour temperature, and higher temperatures indicated higher volatile compounds. The ethanone 2-dimethoxy-1 2-diphenyl- was the most abundant, followed by tetradecane, oxazol-5(4H)-one, 2-phenyl-4-(4-tolylamino) methylene and benzaldehyde while camphene, hexadecane, octane, 4-methyl-, octadecane, 3-ethyl-5-(2-ethylbutyl)-, cyclohexane, 1,2,4-trimethyl-, and beta -phenylpropiophenone had the lowest concentration in the raw BGN seeds based on the mean peak areas at n=2. Although camphene had a low concentration in the raw BGN seeds, it is characterised by a pungent, camphor-like odour used as an additive for food flavouring. In addition, it has anti-inflammatory, anti-allergic, antifungal, and antiseptic properties that can soothe pain in gout, rheumatism, and neuralgic conditions (Capurso *et al.*, 2018).

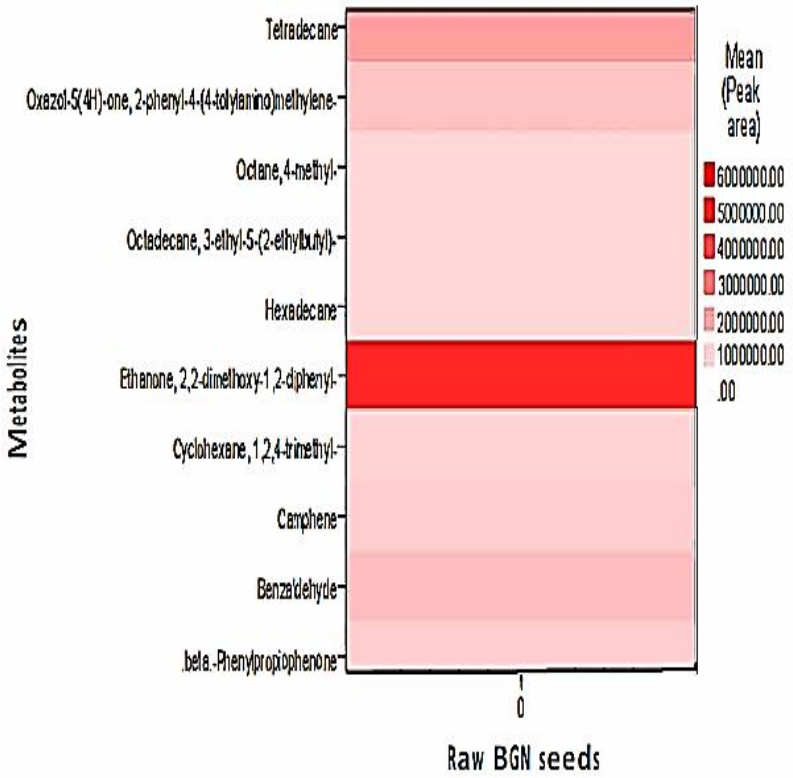


Figure 4.4: Volatiles heatmap for raw Bambara groundnut seeds

Seventeen volatiles were detected in the 36 and 48 h steeped BGN seeds as illustrated in Figure 4.5. The volatiles included pyrimidine-2, 4(1H, 3H)-Dione, 6-amino-1-methyl-5-nitro., pyrazine, 2,6-dimethyl, oxazol-5(4H)-one,2-phenyl-4-(4-1olyamino)methylene, octadecane,5-methylene, morpholine, heptane, 2,2,4,6,6-pentamethyl, fumaric acid,2-methylcyclohex-1-enylmethyl pentadecyl ester, D-Limonene, cyclopentane, methyl, cyclohexane, bicyclo(2,2,1)heptane, 2,2-dimethyl-3-methylene,(1S), benzaldehyde, 3-octen-2-one, (E), 2-methyl-3,4,5,6-tetrahydroppyrazine, 2,2-dimethyl-3-heptene trans, 1-heptanol and .beta.-phenylpropiophenone.

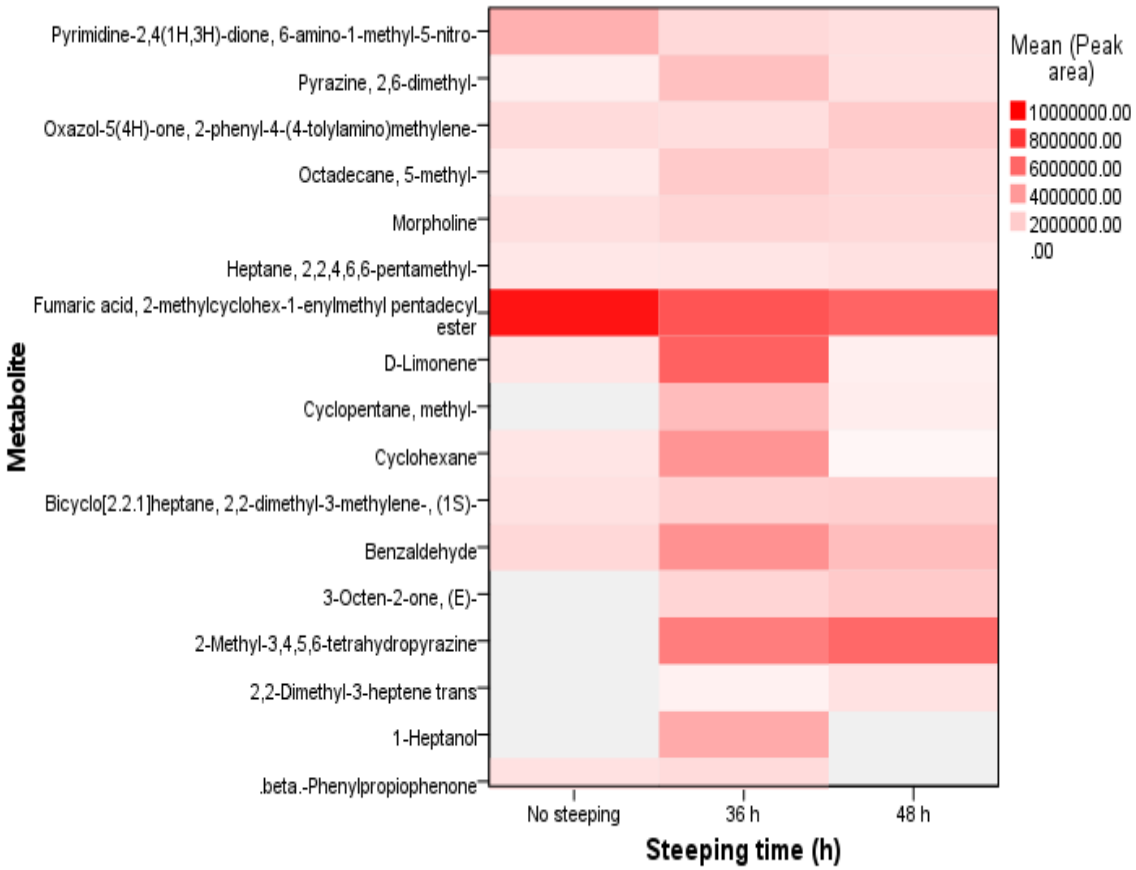


Figure 4.5: Volatiles heatmap for 36 and 48 h steeped Bambara seeds

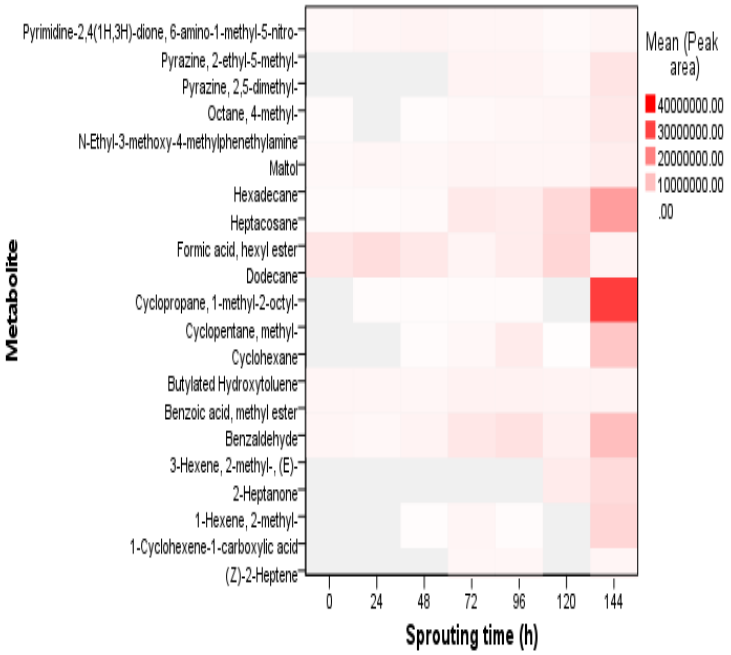
Fumaric acid, 2-methyl cyclohex-1-enyl methyl pentadecyl ester, was the most abundant for the two steeping regimes. The volatiles were more prominent in the 36 h steeped than the 48 h steeped BGN, invariably showing a reduction in most of the volatiles at an increase in steeping time. There was a slight increase in oxazol-5(4H)-one, 2-phenyl-4-(4-tolylamino) methylene- and 2,2-dimethyl-3-heptene trans at 48 h steeping time. Bicyclo [2.2.1] heptane, 2,2-dimethyl-3-methylene-, (1S)- also called camphene was present in the two steeping regimes but highest in the 36 h steeped seeds.

Twelve volatiles were high in 36 h steeped BGN seeds but reduced in the 48 h steeped BGN seeds. The reduction of these volatiles in the 48 h steeped seeds could be that the volatiles were water-soluble and may have been leached out due to increased soaking time (Azarnia *et al.*, 2011; Xiong *et al.*, 2019). 1-heptanol, D-Limonene and beta-phenylpropiophenone were not detected in the 48 h steeped seeds, and this may be due to their insolubility nature in water (FAO, 1997, 2001, 2004; De Gruyter, 2016). 1-heptanol

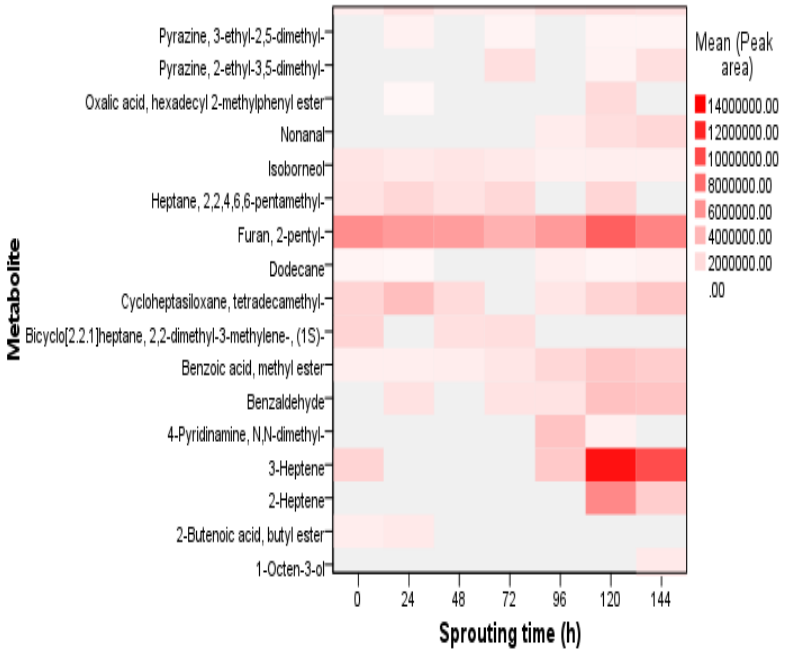
and D-limonene are characterised by beany, musty, woody, citrus odour, while beta-phenylpropiophenone has a pungent, floral, herbaceous odour used in the food and pharmaceutical industries as flavouring agents (FAO, 2001; De Gruyter, 2016; Khrisanapant *et al.*, 2019; Man *et al.*, 2021; Hu *et al.*, 2022). However, oxazol-5(4H)-one,2-phenyl-4-(4-1olyamino) methylene, heptane,2,2,4,6,6-pentamethyl, 3-octen-2-one, (E) and 2-methyl-3,4,5,6-tetrahydroppyrazine were low in 36 h steeped seeds but were dominant in 48 h steeped seeds.

Steeping BGN for 36 h could give desirable volatiles including the pyrazine,2,6-dimethyl with roasted nuts, chocolate, fried potato, coffee-like odour used flavour additive and odorant in foods such as cereals (Müller & Rappert, 2010; Mortzfeld *et al.*, 2020; Ramadan *et al.*, 2021). Fumaric acid is characterised by a fruit-like flavour which is important in industrial applications from feedstock to acidulant in foods and pharmaceuticals (Wiederholte, 2006; Yang *et al.*, 2011; Kirtschig & Schaefer, 2015). D-Limonene is also an additive for foods (sodas, desserts, and candies), cosmetics, and insect repellent to provide a fresh sweet lemony citrus, mint flavour. Benzaldehyde has a sweet, strong almond odour used as an almond flavouring agent for baked goods, scented products, and pharmaceuticals (Valentini *et al.*, 2021; Wen *et al.*, 2021). Bicyclo (2,2,1)heptane, 2,2-dimethyl-3-methylene, (1S), also known as camphene characterised by a mild, oil-camphoraceous aroma used as fragrances and flavouring for food additives, with high antioxidant activity being used in the production of drugs and cosmetics (Felix *et al.*, 2019; Yang *et al.*, 2020).

There were 21, and 14 volatiles detected in the sprouted 36 and 48 h steeped BGN seeds, respectively, as illustrated in Figure 4.6. The volatiles comprising aldehydes, ketones, alcohols, acids, and furans were identified during the sprouting process for the two steeping regimes. Volatiles including cyclopropane, 1-methyl-2-octyl-, hexadecane, cyclohexane and benzaldehyde were higher in the 144 h of sprouting for the 36 h steeped BGN seeds. While furan, 2-pentyl-, 3-heptene, and 2-heptene were significantly higher at 120 and 144 h of sprouting the 48 h steeped BGN seeds. The two steeping regimes had benzaldehyde, benzoic acid, methyl ester, and dodecane as common volatiles detected. However, methyl ester, benzoic acid was reduced in the 48 h steeped BGN seeds, while dodecane remained the same for both steeping regimes. Benzaldehyde, content increased with increasing sprouting times and was highest at 144 sprouting times for the two steeping regimes. Dong *et al.* (2013) noted the increase in benzaldehyde and was considered a key odorant in malts. Benzaldehyde is a bitter almond oil flavour used in savoury food production applications (Marsili, 2016).



(a)



(b)

Figure 4.6: Heatmap volatile metabolites for sprouted Bambara groundnut malt after steeping for (a) 36 h (b) 48 h

Pyrazines including pyrazine, 2,5-dimethyl-, pyrazine, 2-ethyl-5-methyl-, pyrazine, 3-ethyl-2,5-dimethyl- and pyrazine, 3-ethyl-2,5-dimethyl- were detected in the two steeping regimes. Concentrations of the various pyrazines increased during germination; however, no consistent increase in the 48 h steeped BGN seeds, while it was not detected from 0 to 48 h sprouting for the 36 h steeped BGN seeds. The increasing trends of the pyrazines were observed by Kaczmarska *et al.* (2018) in their studies on aroma characteristics of lupin and soybean after germination. Pyrazine is identified by its pungent, roasted hazelnut, roasted, sweetcorn aroma (Marsili, 2016; Parr *et al.*, 2021). It is a nitrogen-containing heterocyclic compound generated through Maillard reactions (Kaczmarska *et al.*, 2018; Wang *et al.*, 2021a). The high concentration of pyrazine towards the end of sprouting could be attributed to the increased heat generation with an increase in germination time since they are thermally induced volatile compounds (Müller & Rappert, 2010; Kaczmarska *et al.*, 2018; Parr *et al.*, 2021). However, the increase has been attributed to the changes in the composition of amino acids and reducing sugars induced by germination (Kim *et al.*, 2021).

The volatile profiling of the raw, steeped, and sprouted BGN seeds showed different volatile profiles from the heat maps. Also noted in the volatiles are the differences in volatile concentration throughout the sprouting process. Changes in the volatiles could be due to the changes in metabolic activities during sprouting, most especially the Maillard reaction and Strecker degradation (Rodriguez-Bernaldo De Quiros *et al.*, 2000; Herrmann *et al.*, 2007) and is dependent on the water imbibed by the seeds, germination and kilning temperature (Herrmann *et al.*, 2007; Channell *et al.*, 2010; Yahya *et al.*, 2014). Thus, steeping for 48 h and sprouting up to 120 h could give the industrial desirable volatile furan, 2 pentyl common in malted grains. It is the main volatile compound in the 48 h steeped seeds and dominant at 120 h. It is characterised by an earthy, beany, nutty pungent aroma important for use as a flavouring agent in tobacco, rum, and chocolate production.

4.7 Conclusions

This study has profiled and identified metabolites present in raw, steeped and sprouted Bambara groundnut. The effect of steeping and sprouting based on times compared to the ungerminated BGN seed exhibited the processing methods as a metabolic process. The metabolite detected showed that the malting process affected the BGN metabolites. FAME reduced with an increase in soaking and sprouting times. Therefore, steeping for 36h and 96 h sprouting could produce healthy FAMEs. Also, there was a reduction in the: acids, sugar, and sugar alcohol of steeped and sprouted BGN in the course of malting. Thus, to maximize the sugar contents of malted BGN seeds, steeping can be done at 36 h and sprouting at 72 h. Generally, amino acids improved during the cause of steeping and

sprouting, showing that malted BGN could be a good source of essential amino acids, and they could be enhanced by malting. However, steeping at 48 h and sprouting for 48 to 72 h could provide a product rich in essential and non-essential amino acids. The volatile components changed with the change in the steeping and sprouting times. Therefore, optimising the production of malted BGN with desirable volatiles would depend on the preferred volatile. However, steeping for 48 h and sprouting for 120 h could produce malted BGN with desirable metabolites that could be beneficial in nutraceutical products and functional ingredients in food production.

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CHAPTER FIVE
PHYSICOCHEMICAL CHARACTERISTICS OF BAMBARA GROUNDNUT SPECIALITY MALTS AND EXTRACT

Abstract

Speciality malts and their extracts are processed following the malting processes as other malts. However, they are treated with high heat and used as a source of additional colour, flavour, and aroma in food production. Speciality malts are mostly produced from cereals grains, and their physicochemical characteristics made them to be sorted for in food production. The objective of this study was to produce speciality malts from BGN seeds and analyse their physicochemical characteristics and metabolites. Bambara groundnut (BGN) speciality malts consisting of base malt (BGN-BM), caramel malt (BGN-CM), roasted malt (BGN-RM), and toasted malt (BGN-TM) were produced from the optimum amylase rich BGN in chapter 3. The BGN-BM was produced by drying sprouted BGN (green malt) at 50^{o}C for 24 h. The BGN-TM was produced from the base malt (dried at 50^{o}C) subjected to 170^{o}C for 30 min. The BGN-CM was produced from the green malt heated to 80^{o}C for 1 h and gradually increased every 20 min to 120^{o}C for 2 h. Roasted malt (BGN-RM) was produced by drying green malt at 180^{o}C for 1 h. Syrups were produced isothermally from the speciality malts. The resultant speciality malts and syrups were assessed for colour, pH, protein, α and β-amylases, total polyphenols, antioxidants, and metabolite profiling. The BGN speciality malts (BGN-BM, BGN-CM, BGN-RM, and BGN-TM) were profiled for volatiles (Headspace Gas Chromatography-mass chromatography, HS-GC-MS), fatty acids methyl esters (Gas Chromatography-Flame Ionization Detection, GC-FID), amino acids, sugars, sugar alcohols and organic acids (Gas chromatography-mass chromatography, GC-MS) metabolites. The colours of the speciality malts and syrups were significantly ($p = 0.000$) different. The BGN speciality malt showed differences in colour with a decrease in lightness (L*) and increased redness (a*) and yellowness (b*). The BGN speciality malts protein ranged from 6.30 to 6.52. The protein content of the BGN speciality malts was significantly different ($p = 0.000$), while the protein content of the syrups was not significantly different. The amylase activities of the BGN speciality malt decreased with the change in kilning temperatures and time. The α and β amylase activities for BGN-BM, BGN-CM, BGN-RM and BGN-TM were 1.01, 0.21, 0.29, 0.15 CU/g and 0.11, 0.10, 0.10, 0.06 BU/g, respectively. The amylase activities of the BGN speciality malts differed for all the malt types. The total polyphenols and antioxidants activities differed for all BGN speciality malts. Twenty-nine volatiles were detected in the speciality malts with the pyrazine, 2,5-dimethyl, more abundant. Fifteen amino acids consisted of seven essential amino acids,

and eight non-essential amino acids were detected. Fatty acid methyl esters (FAME) identified were palmitoleic, oleic, linolelaidic, linoleic and arachidic acid. The sugars, organic acids and sugar alcohols consisted of lactic acid, fructose, sucrose, and myo-inositol. The BGN speciality malts exhibited physicochemical characteristics and metabolites that could benefit household and industrial use.

5.1 Introduction

Household processing such as de-hulling, boiling/cooking, pressure cooking, milling, roasting, fermentation, soaking, and malting are applied to improve physicochemical properties of cereals and legumes (Alain *et al.*, 2007; Subuola *et al.*, 2012; Kavitha & Parimalavalli, 2014; Serventi, 2020)**.** Malting is an inexpensive household food processing that has recently gained attention by researchers to study legumes (Tang *et al.*, 2014; Dueñas *et al.*, 2016; Xue *et al.*, 2016; Serventi, 2020; Viktorinová *et al.*, 2020). Malting consists of three simple steps: steeping, sprouting and drying under controlled conditions (Erba *et al.*, 2019; Lemmens *et al.*, 2019).

Malting of legumes has been reported to encourage an increase in free amino acids and vitamins by the modification of the functional properties of seed physical and chemical components (Dueñas *et al.*, 2005; Owuamanam *et al.*, 2014; Mäkinen & Arendt, 2015; Marcela, 2017; Manickavasagan & Thirunathan, 2020). In addition, malting promotes hydrolytic enzymes, which are not present in un-germinated grains (Aguilera *et al.*, 2013; Kavitha & Parimalavalli, 2014; Dziki & Gawlik-Dziki, 2019; Chinma *et al.*, 2021). Due to the activation of the hydrolytic enzyme, the malting process (soaking, sprouting, and kilning) gives malted grains their characteristic colour, taste, flavour, and nutritional components (Murugkar, 2014; Boukid *et al.*, 2019; Asuk *et al.*, 2020).

The final step of malting, kilning, is a biochemical process applied to cereals and legumes to enhance their physicochemical properties. The kilning temperature and time are increased to obtain desired malt properties such as enzymes, moisture removal for stabilization, raw flavours removal, malty flavours, and colour development (Lloyd, 1988; Samaras *et al.*, 2005; Carvalho *et al.*, 2014, 2016; Sharma *et al.*, 2020). During the kilning process, the reaction of sugars and amino acids promotes melanoidins and reductones through the Maillard reaction (Woffenden *et al.*, 2001; Samaras *et al.*, 2005; Coghe *et al.*, 2006; Koren *et al.*, 2019). The melanoidins formed are responsible for the antioxidant potential of the speciality malt types (Bamforth, 2006; Coghe *et al.*, 2006; Carvalho *et al.*, 2014; Koren *et al.*, 2019). For example, the dark speciality malts Maillard reaction products (Melanoidins) are significant antioxidants that increase with increasing malt colour due to

changes in kilning temperature and time (Woffenden *et al.*, 2001; Coghe *et al.*, 2004c; Sharma *et al.*, 2020).

Producing speciality malts comes with different drying temperatures (Coghe *et al.*, 2006; Cortés *et al.*, 2010; Gąsior *et al.*, 2020). Base malts are produced at a low temperature between 50 to 80°C for their high degree of diastatic power. The base malt mostly precedes other speciality malts from roasted, toasted, and caramel malts used in food processing for various benefits (Coghe *et al.*, 2004c, a, 2006). Caramel, roasted, and toasted malt are termed speciality malt because they are produced primarily because of their characteristic high antioxidant, colour, and flavour (Coghe *et al.*, 2004b,c; Vandecan *et al.*, 2011; Davies, 2016; Kábelová-Ficová *et al.*, 2017; Gąsior *et al.*, 2020). Generally, speciality malts and their extracts (syrup) add sensory benefits to the final product by enhancing their colour, flavour, and taste (Mäkinen & Arendt, 2015b; MacLeod & Evans, 2016; Rögner *et al.*, 2021).

Barley is the most used in malt production, and it's majorly used in the brewing and food industries (Guido & Moreira, 2013). Although barley malt is commonly used, other cereals and legumes are also malted to access their nutritional values (Dziki & Gawlik-Dziki, 2019). Mung beans, soybeans, cowpea, black beans, lentils, chickpea, and Bambara groundnut are some of the legumes that have been sprouted and studied for their nutrition, physicochemical and functional characteristics (Mayes *et al.*, 2019).

Bambara groundnuts (BGN) is not a commonly known legume crop in many parts of the world. However, it is categorized as the third most crucial legume in Africa, after peanuts and cowpeas (Ahmad *et al.*, 2015; Diedericks *et al.*, 2020). Sustainable food experts have gained interest in BGN because it is an underutilized and nutritious crop (Drewnowski, 2020). BGN is an indigenous plant cultivated in Africa on a small scale by subsistence farmers (Nwokolo, 1996; Oyeyinka *et al.*, 2017). It is categorized as a good quality protein food containing substantial-high proteins, carbohydrates, fats, and minerals (Diedericks *et al.*, 2020; Manickavasagan & Thirunathan, 2020).

Following the malting process, BGN seeds' physicochemical, functional, thermal, health-promoting, and nutritional properties greatly improved while reducing their anti-nutritional factors (Manickavasagan & Thirunathan, 2020; Chinma *et al.*, 2021). Abba *et al.* (2018) noted that malting BGN improved its protein content. Value-added snacks, weaning, ready-to-eat, and composite products have been made from malted BGN seeds and have shown improvement over the un-malted seeds (Jideani, 2016; Awobusuyi & Siwela, 2019; Agu *et al.*, 2020). The nutritional and functional characteristics of malted BGN in the production of *Okpa*, composite biscuit, flours, and infant formula have been carried out (Akpapunam *et al.*, 1996; Nzelu, 2016; James *et al.*, 2018a; Ding & Feng, 2019; Agu *et al.*,

2020). Bambara groundnut was subjected to steeping (36 and 48 h) and sprouting (0 to 144 h) at different times to study their physicochemical characteristics and metabolite components in the previous chapters. In this study chapter, the amylase-rich BGN malt was produced by steeping for 36 h and sprouting for 96 h, which was used to produce speciality malts. However, no documented study reports the physicochemical characteristics of BGN speciality malts and syrups products. Thus, this work investigated the production of BGN speciality malts, syrups, their physicochemical characteristics, and metabolites.

5.2 Materials and Methods

5.2.1 Source of materials, reagents, and equipment

Amylase rich BGN malt produced from 36 h steeped seeds and sprouted at 96 h was used to produce BGN speciality malts. Chemicals and reagents were of analytical standards. Alpha and beta-amylase kits were from Megazyme Ltd, Ireland. All equipment was from the Department of Food Science and Technology and Oxidative Stress Research Centre, Cape Peninsula University of Technology, Cape Town, South Africa. Dumas nitrogen analyzer LECO CN 628 (Leco Corp., St Joseph, MI, USA), the centrifuge (Avanti® J-E centrifuge JSE111330, Beckman coulter Inc., USA), and Thermos Scientific Multiskan plate reader spectrophotometer (Thermo Scientific, USA). Others are the pH meter (Hannah checker pH meter, Model HI1270), a water bath, Colour Flex EZ (Model TC-P III-A, Tokyo Denshoku Co., Ltd., Japan), and an Excalibur Food Dehydrator (Excalibur, Sacramento, CA, USA). Figure 5.1 outlines the analyses carried out in this chapter.

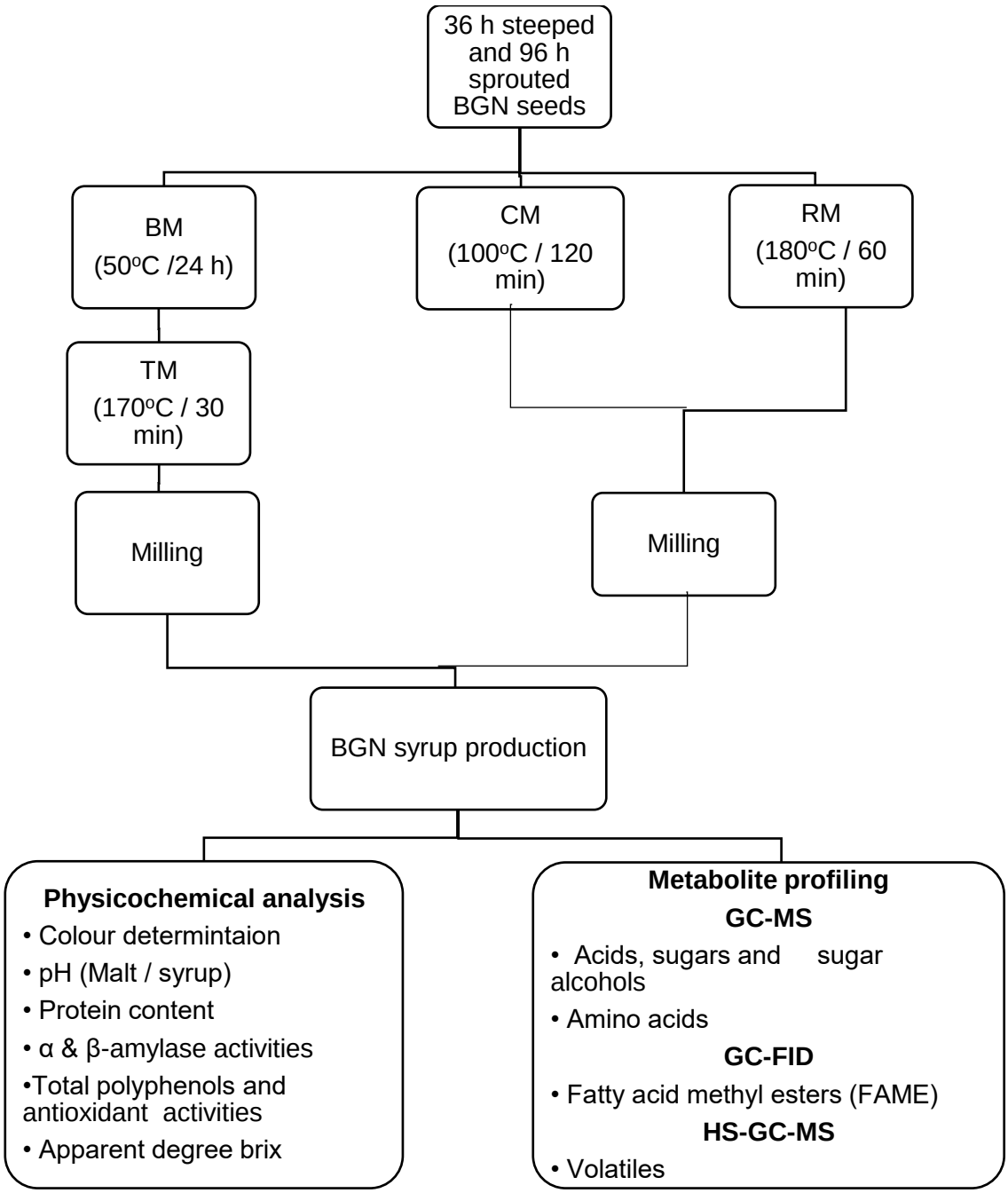

Figure 5.1: Chapter 5 outline

GC-MS: Gas Chromatography-Mass Spectrometry, GC-FID: Gas Chromatography-Flame Ionization Detection, HS-GC-FID: Headspace Gas Chromatography-Flame Ionization Detector

5.2.2 Production of Bambara groundnut speciality malts

The production of the BGN speciality malts was carried out following the method described by Michael (2014), with some slight differences in processing temperatures. The process flow of the speciality malts and their syrups is as shown in Figure 5.2. The amylase rich 36 h steeped, 96 h sprouted green BGN malt was divided into four parts of 100 g each and dried at different temperatures to produce BGN malt varieties. BGN Base malt (BM) was produced from sprouted BGN (green malt) by drying at 50^{o}C for 24 h in a hot air Excalibur Food Dehydrator (Excalibur, Sacramento, CA, USA). Toasted malt (TM) was produced from the base malt (dried at 50^{o}C) by subjecting it to a higher temperature of 170^{o}C for 30 minutes.

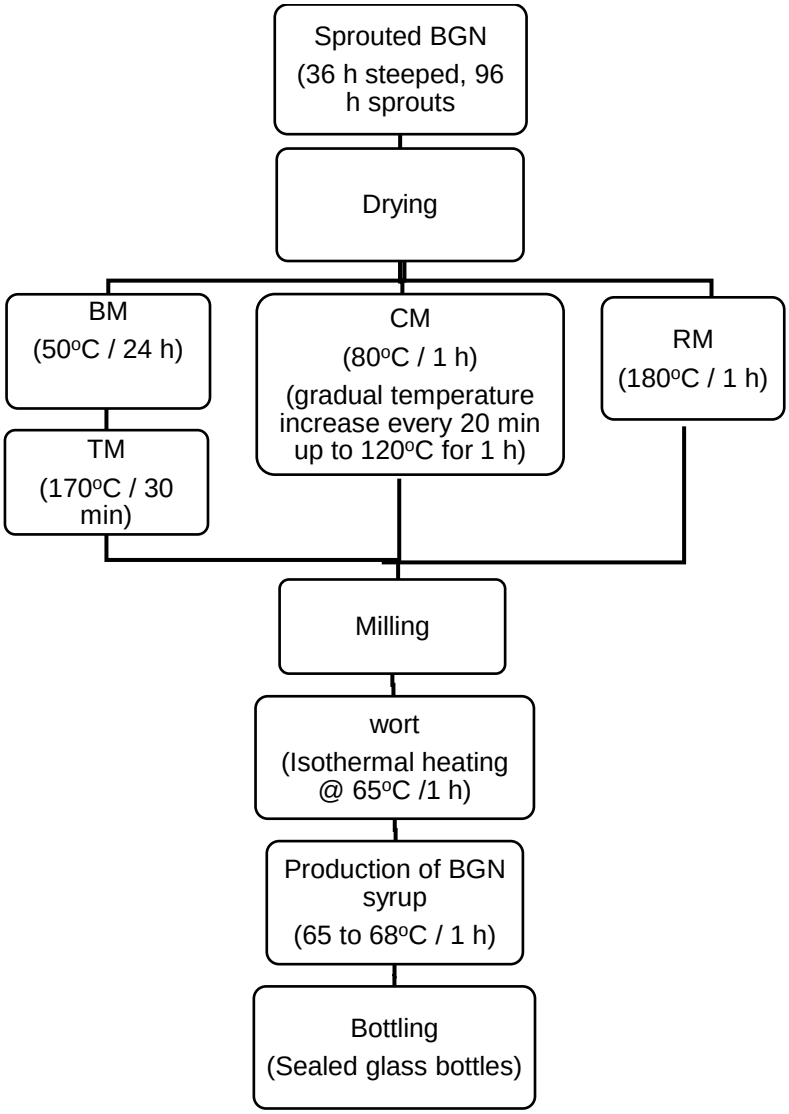

Figure 5.2: Process flow of Bambara groundnut speciality malts

Finally, roasted malt (RM) was produced by drying green malt at 180^{o}C for 1 h in the baking ovens twin deck (Mac bake). The malts were packed in zip lock bags and stored at -18^{o}C in the baking ovens twin deck (Mac bake). The caramel malt (CM) was produced from the green malt at an initial temperature of 80^{o}C for 1 h, then gradually increased the temperature by 20^{o}C every 20 min up to 120^{o}C for 2 h in the baking ovens twin deck (Mac bake) until analysis for physicochemical (pH, colour), protein, total polyphenol, antioxidant, amylase activities and metabolites.

5.2.3 Bambara groundnut malt syrup production

The base, caramel, roasted and toasted (BM, CM, RM, and TM) BGN speciality malts were milled using the Waring laboratory blender to obtain a fine powder. Fifty grams of base malt, and a ratio of base malt of 20% to caramel, roasted and toasted malts of 80% were extracted with 350 mL of distilled water in 500 mL beakers following the method of Krebs *et al.* (2020). Mashing was conducted isothermally at 65^{o}C using the laboratory magnetic hotplate stirrer (ONiLAB USA, model no MS-H-ProA) with continuous stirring for 60 min. Fifty millilitres (50 mL) of distilled water at 65^{o}C was added after 30 min, and mashing was continued isothermally for 30 min at 65^{o}C. The malt extracts were afterwards cooled to room temperature and adjusted to 450 g with distilled water. The extracts were centrifuged for 15 min at 4400 rpm, and the supernatants were transferred into 1000 mL beakers. The extract was heated between 65 to 68^{o}C for 60 min to make syrups and cooled to room temperature. The BGN malt syrups were packaged in glassware sample bottles stored at -18°C until analyzed for pH, colour, Brix, protein content, total polyphenol content, antioxidant, and amylase activities.

5.3 Bambara Groundnut Speciality Malts and Syrups Physicochemical Analysis

5.3.1 Colour determination of speciality Bambara groundnut malts and syrups

The Bambara groundnut speciality malt and their respective colour measurements were analysed using Colour Flex EZ (Hunter Lab, Reston, VA, USA), 25 mm aperture set for daylight illumination D65, and 10° standard observer angle following the Method of Sofi *et al.* (2020). The instrument's calibration was done using standard black (L* = 8.47, a* = -0.96, b* = 2.79) and white (L*=8.47, a*= -0.96 b*= 2.75) tiles. The colour measurement was in triplicates by measuring 5 g of the samples into a glass sample cup (Hunter Lab 04720900, 6.4 cm) with an internal diameter of 6.4 cm following the method by Panghal *et al.*(2019). The Commission Internationale de l'Eclairage's (CIE) L*a*b* was used to measure the colour parameters L* (0 = black and 100 = white), a* (− a* = greenness, and + a* = redness) and b* (− b* = blueness and + b* = yellowness). The chroma and hue

were calculated using the method of Medhe *et al.* (2019), as shown in equations 5.1 and 5.2.

$$C = \sqrt{a^{*2} + b^{*2}} \qquad \text{Equation 5.1}$$

Where C = Chroma; a^{*2} = redness; b^{*2} = greenness

$$h^{o} = \tan^{-1}\left(\frac{b^{*}}{a^{*}}\right) \qquad \text{Equation 5.2}$$

Where h^{o}= Hue angle; a^{*2} = redness; b^{*2} = greenness

5.3.2 pH determination of speciality Bambara groundnut malts and syrups

Following the Atudorei et al. (2021) method with some differences, 10 g milled BGN speciality malt (BM, CM, RM, and TM) were separately mixed with 40 mL distilled water in a 50 mL centrifuge for 5 minutes using a vortex mixer. The mixtures were kept at room temperature for 1 h and centrifuged at 1500 × g for 10 min. The decanted liquid pH was measured in triplicate at room temperature using a laboratory pH meter (Hannah checker pH meter, Model HI1270), calibrated with buffers 4 and 7.

5.3.3 Determination of Bambara groundnut speciality malts and syrups protein content

Bambara groundnut speciality malts and syrups' crude protein was determined using the LECO CN 628 Dumas nitrogen analyser (Leco Corp, St Joseph, MI, USA). Five blanks, EDTA standard, and ProNutro control sample were first analysed and then samples in duplicate. The samples were measured in triplicate to the value of 0.09 mg, wrapped and tightly folded in tin foil cups P/N: 502-186-200. The samples' combustion was carried out in pure oxygen at a temperature of 950^{o}C in the reactor consisting of the combustion catalyst. A gaseous mix containing carbon dioxide, water, and nitrogen (CO2, H2O, NO, NO2) was formed during the fast combustion reaction. The designated columns then absorbed the gases, removed oxygen, and converted nitrogen oxides into nitrogen. The residual carbon dioxide (CO_2) and water were extracted through a thermal conductivity column carried by helium gas. The Dumas Nitrogen analyser measured the nitrogen content. The crude protein was calculated by multiplying the measured nitrogen by the protein factor of 6.25 expressed in percentage following Awobusuyi & Siwela (2019) methods.

5.4 Determination of apparent degree Brix (°Brix) of Bambara groundnut syrups

Ofoedu *et al.* (2020) method was used to measure the total soluble sugar of the syrups at a temperature of 20°C with a handheld KERN-SOHN refractometer (KERN ORA 10 BA/BB Kern & Sohn, GmbH, Germany). First, the standardization of the handheld refractometer was carried out with distilled water at 20°C until the Brix value read zero. Then, one drop of the BGN syrups was dropped on the lens (sensitive surface) using plastic filling pipettes and measurements were taken. Finally, the total sugar contents (°Brix) were read from the refractometer scale in triplicate.

5.5 Determination of α- and β-Amylases Activities of Bambara Groundnut Speciality Malt

The α and β- amylase activities of the BGN speciality malts and syrups were determined following the method of Montanuci *et al.* (2017), stated in the instructions of the enzyme kits. The α- and β-amylase enzymes were determined through the enzymatic Ceralpha Method (K-CERA, Megazyme) and the enzymatic kit Beta-amylase (Megazyme, K-BETA3), respectively as detailed in sections 4.4.1 and 4.4.2. All analyses for enzymatic activity were done in triplicate.

5.5.1 Alpha-amylase assay procedure (Ceralpha method)

The milled BGN speciality malt and syrups of 3.0 g were measured separately into 50 mL conical flasks. To each flask, 20 mL of extraction buffer solution of pH 5.4 was added, and the contents of the flask were stirred vigorously using the vortex mixer. The samples were then allowed to extract for 20 min at 40°C in the incubator and occasionally stirred with a vortex mixer. After extraction, 25 mL of each sample were measured into 50 mL centrifuge tubes and centrifuged using the Centrifuge 5810R at 1,000 g for 10 min. Finally, the sample extracts were decanted into 25 mL centrifuge tubes for the assay procedure.

The assay was carried out by measuring 0.2 mL aliquots of Megazyme un-buffered amylase HR reagent into 25 mL centrifuge test tubes. It contains blocked p-nitrophenyl maltoheptaoside (BPNPG7, 54.5 mg) and thermostable α-glucosidase (125 U at pH 6.0). The two were pre-incubated at 40°C for 5 min. The 0.2 mL sample extracts were also pre-incubated at 40°C for 5 min. The pre-incubated 0.2 mL of the samples were added directly to the tubes' bottom containing the 0.2 mL of the amylase HR reagent solution. These were incubated at 40°C for 20 min immediately. At the end of the 20 min incubation period, exactly 3 mL of stopping reagent (10 g of tri-sodium phosphate in 1 L of distilled water pH adjusted to 11.0) was added. The contents of the tube were vigorously stirred using the

vortex mixer. The absorbance of the solutions was read in triplicate using the Thermo Electron Corporation Multiskan Spectrum set at 400 nm against distilled water.

5.5.2 Beta-amylase assay procedure (Betamyl-3 method)

Samples (BGN speciality malt and syrups) of 0.5 g were weighed into 25 mL centrifuge tubes. A five mL extraction buffer (Tris/HCl 25 mL, 1 M, pH 8.0 plus disodium EDTA of 20 mM and sodium azide of 0.02% w/v diluted in distilled water) was added. The enzymes were allowed to extract for one hour at room temperature, with repeated stirring on the vortex mixer. The mixtures were centrifuged using the Eppendorf Centrifuge 5810/5810 R at 2,000 *g* for 10 min. Immediately after centrifugation, 0.2 mL of the filtrate were added to 4 mL of the dilution buffer containing MES dilution buffer 48 mL, 1 M, pH 6.2 plus disodium EDTA 20 mM, BSA 10 mg/mL, and sodium azide of 0.09% w/v. This mixture was then used for the assay of β-amylase activities.

The assay of the β-amylase was done by dispensing an aliquot of 0.2 mL of the diluted samples into the bottom of 25 mL centrifuge tubes. The tubes were pre-incubated at 40^{o}C for 5 min. After incubation, 0.2 mL of pre-incubated Megazyme Betamyl-3 substrate solution containing p-nitrophenyl-β-D-maltotrioside (PNPβ-G3) plus β-glucosidase (50 U) and stabilisers were added to each diluted sample and stirred on the vortex mixer. These mixtures were incubated at 40^{o}C for 10 min. At the end of the 10 min incubation, 3.0 mL of the stopping reagent (10 g of Tris buffer (Megazyme cat. No. B-TRIS500) in 900 mL of distilled water, pH adjusted to 8.5 was added. The contents were stirred using a vortex mixer. The absorbance of the solutions and the reagent blank reading was at 400 nm. The absorbance was read against distilled water using a Thermo Scientific Multiskan microplate spectrophotometer.

5.6 Determination of Total Polyphenols and Antioxidants Activities of Bambara Groundnut Speciality malt

The Folin–Ciocâlteu reagent (FCR), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay methods used for the determination of phenolic activities and antioxidants were followed (Xu and Chang, 2008; Nemzer *et al.*, 2019; Rico *et al.*, 2020) as detailed in sections 4.5.1 to 4.5.2.

5.6.1 Total polyphenols content determination by Folin–Ciocâlteu Reagent (FCR) assay

The analysis uses the Folin-Ciocalteu reagent (FCR) with gallic acid as the standard to quantify total polyphenols in samples. The phenolic contents were determined by weighing

500 mg of each sample (BGN speciality malt and syrups) into screw-cap tubes. The samples' extraction was carried out with 10 mL of 70% methanol mixed with 0.1% HCL. The mixture was mixed with a vortex mixer. The samples were then centrifuged using the Eppendorf Centrifuge 5810/5810 R at 4000 g, 21^{o}C for 5 min. The supernatant was analysed using the Folin–Ciocalteu assay. Twenty-five microliters of the sample's supernatant were mixed with 125 µl of 0.2 M Folin–Ciocalteu reagent and 100 µl of 7.5% Na_2CO_3 solution in a 96-well transparent plate. The absorbance was read in triplicate with a Thermos Scientific Multiskan microplate spectrophotometer reader (734 nm at 25^{o}C) after a 2 h incubation period. The standard calibration curve was constructed with 40 mg gallic acid (Sigma Cat Nr: G7384). The results were expressed as mg Gallic acid equivalents (GAE/g).

5.6.2 Antioxidant activities determination by ferric reducing antioxidant power (FRAP) assay method

Bambara groundnut speciality malt and syrups of 500 mg were weighed into 50 mL screw-cap tubes. Ten millilitres of 70% methanol (containing 0.1% HCl) were added to the samples in the screw-cap tubes. The samples were mixed with a vortex, then centrifuged at 4000 rpm for 5 minutes and the supernatants (10 µL each) were pipetted into microplate wells in triplicates. Three hundred microliters (300 µL) of the FRAP reagent were added to each sample in the microplate wells. Ascorbic acid was the standard, and distilled water was the blank. The samples were incubated for 30 min at 37^{o}C, and absorbance was read at 593 nm. The Thermos Scientific Multiskan microplate spectrophotometer was used for reading absorbance. The results were expressed as mg ascorbic acid equivalents (AAE)/g.

5.6.3 Antioxidant activities determination by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method

The samples' free radical scavenging ability was determined using the DPPH radical (25 mg/l) in 70% methanol. Each of the samples was mixed with 0.275 mL DPPH solutions. The samples and standards were incubated at 37^{o}C for 30 min in the dark, and absorbance reactions were read at 517 nm. The Thermos Scientific Multiskan microplate spectrophotometer was used for reading absorbance. The standard was Trolox, and results were expressed as µmole Trolox /g.

5.7 Metabolite Profiling of Bambara Groundnut Speciality malt

The metabolite profiling was carried out on the Bambara groundnut speciality malt. The sugars, sugar alcohols and organic acids, and amino acids were profiled by capillary gas

chromatography-mass spectrometry (GCMS) (Frank *et al.*, 2007, 2009, 2011; Shu *et al.*, 2008; Jom *et al.*, 2011). A gas chromatography-flame ionization detector (GC-FID) was used to analyse the fatty acid methyl esters (FAME) (Salmerón *et al.*, 2015). A headspace gas chromatography-flame ionization detector (GC-FID) was used to analyse the fatty acid methyl esters (FAME) and volatile compounds (AOAC, 2005)

5.7.1 Determination of Fatty Acids Methyl Esters (FAME) and hydrocarbons by Gas Chromatography-Flame Ionization Detection (GC-FID)

The analysis of fatty acid and hydrocarbons was carried out by extracting and converting the BGN speciality malts lipids into fatty acid methyl esters (FAME). The extraction was carried out using diethyl ether and petroleum ether in methanol. A model Agilent 7890A gas chromatography (GC) coupled with Flame Ionization Detection (GC-FID) was used for detection according to the AOAC (2005) method 996.06 with some modifications.

The BGN speciality malts of 1.5 mg were weighed into the separate 70 mL test tubes to digest. The tube's contents were thoroughly mixed with 100 mg of pyrogallic acid, 2 mL internal standard solution of 5mg/mL undecanoic acid dissolved in hexane and 2 mL ethanol. Immediately after mixing, 10 mL of 32% HCL was mixed into each tube. The tubes were then placed in a 70–80^{o}C water bath for 40 min and contents mixed every 10 min. After digestion, the tubes were removed and allowed to cool to room temperature. The 25 mL diethyl ether was added to each tube and shook for 5 min for extraction. Petroleum ether of 25 mL was further added and shook for 5 min. After separating the two layers, the clear upper layer was decanted into 150 mL beakers, and ether was evaporated in the fume hood to dryness.

Derivatisation of the samples was carried out by reconstituting the residues in 3 mL chloroform and diethyl ether. The solutions were transferred into 10 mL tubes and evaporated under the Nitrogen stream to dry. Immediately after drying, 2 mL of 2% H_2S0_4 in methanol reagent and 1 mL toluene were added. The tubes were tightly closed and placed in the incubator at 100^{o}C for 45 mins, then cooled to room temperature. After cooling, 5 mL distilled water and 1 mL hexane was added and thoroughly shaken using the vortex mixer for 1 min. The layers were left to separate, and the top layers were carefully transferred to 20 mL test tubes. Approximately 1 g anhydrous Na_2S0_4 was added to each tube to have a clear solution. The clear solutions were then transferred into 2 mL clear vials, and GC analysis was carried out. Fatty acids were identified by comparing their retention times to the retention times of the standard.

5.7.2 Sugars, acids, and sugar alcohols determination by Gas Chromatography-Mass Spectrometric (GC-MS)

Sugars, sugar alcohols, and organic acids were analysed using GCMS by measuring 1 mL of 70% methanol (MeOH), then adding approximately 100 mg of the BGN speciality malts and extracting at 45°C in the oven for 3 hours. The extracted samples of 130 µl were dried completely with a gentle stream of nitrogen and derivatised with 100 µl of methoxamine at 40^{o}C for 2 h. Then 30 µl of N, O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) was added and derivatised at 60^{o}C for 30 minutes. Finally, the samples were transferred into 2 mL GC vials, and 1 µl was injected onto the GC-MS in spitless mode.

Separation was performed on a gas chromatograph (Trace 1300, Thermo Scientific) coupled to a mass spectrometer (TSQ 8000, Thermo Scientific). The carbohydrates were separated on a non-polar capillary column Rxi-5Sil MS (30 m, 0.25 mm ID, 0.25 µm film thickness). Helium was used as the carrier gas at a 1 mL/min flow rate. The injector temperature was maintained at 250^{o}C. The oven temperature was 80^{o}C for 1 min and ramped up to 300^{o}C at a rate of 7^{o}C/min and held for 2 min.

5.7.3 Determination of amino acids by Gas Chromatography-Mass Spectrometry (GC-MS)

Following the method of Stenerson (2011) with a little difference, 3 mL of 6M HCl was added to *ca.* 500 mg of the BGN speciality malts (BM CM, RM and TM). They were hydrolysed for 24 hours at 110^{o}C, cooled down to room temperature and diluted at a ratio of 1:9 with 70% methanol (v/v). Next, 100 µl was transferred into a 2 mL tube and dried completely under a gentle stream of nitrogen. Then, the samples were reconstituted and derivatised with 30 µl silylation reagent N-tert-butyldimethylsilyl- N-methyl trifluoroacetamide (MTBSTFA) and 100 µl acetonitrile at 100^{o}C for 1 hour. After which, they were cooled down to room temperature and injected into the GC-MS instrument for analysis.

Component separation was performed on a gas chromatograph (Trace1300, Thermo Scientific) coupled to a TSQ8000 mass spectrometer (Thermo Scientific). The GC-MS system was connected to a TriPLUS autosampler. Amino acids were separated on a Rxi-5Sil MS (30 m, 0.25 mm ID, 0.25 µm film thickness) capillary column. Helium was used as the carrier gas at a 1 mL/min flow rate. The injector temperature was maintained at 250°C. In addition, 1µl of the sample was injected in spitless mode. The oven temperature was programmed to 100^{o}C for 1 minute and ramped up to 300^{o}C at a rate of 15^{o}C/min and held for 6 minutes. The Agilent mass spectrometer detector (MSD) was operated in scan mode, and the source and quad temperatures were maintained at 250^{o}C and 150^{o}C, respectively. The transfer line temperature was maintained at 250^{o}C. The mass

spectrometer was operated under electron impact (EI) mode at ionization energy of 70eV by scanning from 35 to 650m/z.

5.7.4 Determination of volatile compounds by Headspace Gas Chromatography-Mass Spectrometry (HS-GC-MS)

The headspace gas chromatography-mass spectrometry (HS–GC–MS) analyses were performed using a model Agilent 7890B Gas Chromatography–5977A coupled with Mass Spectrometer detector system (Santa Clara, CA, USA) with a split-less injector suitable for GC analysis by following the method of (Salmerón *et al.*, 2015) with some differences. The Agilent J&W GC HP-5ms capillary column of 30 m x 0.25 mm x 0.25 µm was used to separate the volatiles. The carrier gas was Helium, with a 0.6 mL/min flowrate. Two hundred and fifty microlitres of the speciality malts volume were injected with a split ratio of 50:1 and weighed into 10 mL glass headspace vials covered with silicon septum with a purge flow of 3 mL/min and screw-capped. The oven temperature was 50°C held for 5 minutes, increased at 10°C/min to 200°C and held for 5 minutes with a running time of 25 min. The injector temperature, pressures, and volume were set at 240^{o}C, 2.6149 psi and 250 µl, respectively. The incubation temperature and time were set at 120^{o}C and 300 sec, respectively. The samples were then run concurrently.

The compounds were identified through Wiley mass spectral (MS) library and Golm metabolome database search. The volatile compounds identification was by comparing the mass spectra with the spectra of the reference compounds in both the Wiley MS library and verified based on mass spectra obtained from the literature. The volatile results were provided based on the compounds' quality and peak area counts.

5.7.5 Identification of metabolite compounds

Identification of BGN speciality malt constituents was done by comparing retention times and mass spectra with reference compounds. Also, by comparing mass spectra with the entries of the National Institute of Standards and Technology mass spectra library NIST02 and the GOLM metabolome database (Shu *et al.*, 2008; Frank *et al.*, 2009; Jom *et al.*, 2011; McGough *et al.*, 2012).

5.8 Statistical Data Analysis

All results were reported as mean ± standard deviation of three independent trials. Multivariate analysis of variance (MANOVA) was used to establish differences between treatments. Duncan’s multiple range test was used to separate means where significant

differences existed (IBM SPSS version 26, 2019). Kruskal Wallis test was used to test the distribution of protein across categories of sprouting time (h).

5.9 Results and Discussion

5.9.1 Colour characteristics of Bambara groundnut speciality malts and syrups

The CIE L*a*b* colour space coordinates, chroma and hue of the BGN speciality malts consisting of base (BM), caramel (CM), roasted (RM) and toasted (TM) malts are shown in Table 5.1. The lightness (L*) and the hue angle (h^o) decreased from 74.12 to 45.98 and 71.54 to 53.90 for the BGN speciality malt types. The redness (a*), yellowness (b*) and chroma increased for the BGN speciality malts, 3.96 to 16.44, 11.85 to 22.68 and 12.50 to 28.03, respectively. There was a significant difference across the lightness, redness, yellowness, chroma and hue for all the speciality malt. As seen by the physical eye, the colour of the speciality malt was as shown in Figure 5.3.

The colour change has been attributed to the non-oxidative Maillard reaction due to heat (Coghe *et al.*, 2004a). The reaction between reducing sugars and amino acid contents of malted grains consists of complex reactions generally termed Maillard reaction (Kramer, 2015). The reactions are important mechanisms of non-enzymatic browning during heat processing of malt (Woffenden *et al.*, 2002b). The factors affecting the degree and magnitude of the Maillard reaction are temperature, time, water activity, concentration (Coghe *et al.*, 2004a). These factors affect the end product and give the products their characteristic colour, flavour and anti-oxidative activity, essential in industrial food products (Lekjing & Venkatachalam, 2020).

Table 5.1 Colour characteristics of Bambara groundnut speciality malt[1]

BGN speciality malt	L*	a*	b*	C	h^o
Base	74.12 ± 0.29^a	3.96 ± 0.71^a	11.85 ± 1.24^a	12.50 ± 1.30^a	71.54 ± 2.63^a
Caramel	74.24 ± 0.26^a	4.76 ± 0.86^a	15.31 ± 0.26^a	16.04 ± 0.33^a	72.74 ± 2.98^a
Roasted	63.91 ± 0.45^b	9.87 ± 0.52^b	22.41 ± 2.55^b	24.51 ± 2.23^b	66.05 ± 3.06^b
Toasted	45.98 ± 0.27^c	16.44 ± 0.63^c	22.68 ± 2.99^b	28.03 ± 2.80^b	53.90 ± 2.48^c

[1]Values are mean of triplicates ± standard deviation, mean values in the same column with different superscript letters are significantly different (P ≤ 0.05). BGN- Bambara groundnut, L*- Lightness; a*- Redness, b*- Yellowness, C- Chroma, h– Hue angle

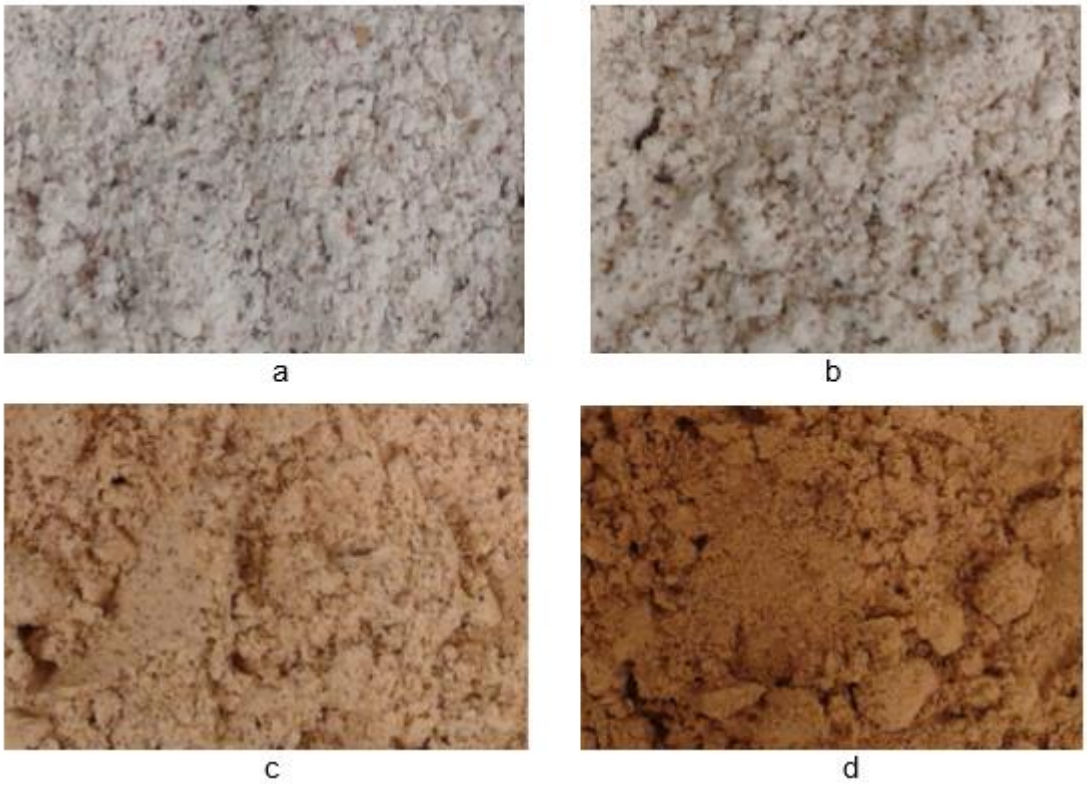

Figure 5.3: Bambara groundnut speciality malts (a) Base malt, (b) Caramel malt, (c) Roasted malt and (d) Toasted malt

The colour of the malt is greatly affected by the temperature and time (O'Rourke, 2002a). Yahya *et al.* (2014) reported a similar result in the production of barley and malt roasting operations where the product became darker as temperature increased above 150^{o}C, showing lightness (L*) reducing from 75-40. Furthermore, the evaluation of the colour coordinates in the study of barley speciality malt features showed a colour difference, whereby there was a decrease in lightness (L*) at high temperatures, while the redness (a*) and yellowness (b*) increased at higher temperatures (Vandecan *et al.*, 2011). Studies on the development of Maillard reaction during production of roasted (caramel) malt demonstrated that the colour formation is dependent mainly on the time and temperature of kilning (Coghe *et al.*, 2004c, 2006; Mayer *et al.*, 2016). However, the colour changes were attributed to the measure of shorter chain melanoidins or caramelisation by conversion to darker coloured malt with increased temperatures (Carvalho *et al.*, 2016).

The base, caramel, roasted and toasted malt syrups lightness are indicated in Table 5.2. The chroma and hue angle (h^{o}) for the base, caramel, roasted and toasted malt syrups

ranged from 8.68 to 21.75 and 59.20 to 73.53^{o}, respectively. The hue angle (h^{o}) of the BGN syrups represents the red to yellow colour range (0^{o}-90^{o}). There is, however, a significant ($p = 0.000$) difference in the speciality malts syrups lightness, redness, yellowness, and chroma except for hue.

Table 5.2 CIE L*a*b*, Chroma and hue for the Bambara groundnut speciality malt syrups[1]

	Bambara groundnut speciality malt syrups			
Colour characteristics	**Base malt**	**Caramel malt**	**Roasted malt**	**Toasted malt**
L*	49.56 ± 0.15^{a}	52.77 ± 0.07^{b}	41.43 ± 0.32^{c}	28.55 ± 0.69^{d}
a*	8.07 ±1.63^{a}	7.78 ± 1.46^{a}	9.51 ± 2.64^{a}	2.52 ± 0.99^{b}
b*	15.82 ± 1.41^{a}	20.28 ± 0.76^{b}	16.22 ± 3.12^{a}	8.30 ± 1.40^{c}
Chroma	17.82 ± 1.10^{a}	21.75 ± 0.70^{b}	19.04 ± 1.75^{a}	8.68 ± 1.62^{c}
Hue angle	62.94± 5.10^{ab}	69.03 ± 3.90^{ab}	59.20 ± 11.47^{a}	73.53 ± 3.66^{b}

[1]Values are mean ± standard deviation of triplicate values, mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$). L*- Lightness; a*- Redness, b*- Yellowness

The higher colour values signify the BGN speciality malt syrup colour's intensity. The reduction in lightness of the syrups from base malt syrup to toasted malt syrup was attributed to the Maillard reaction developing Maillard reaction products during heating and caramelization (Murevanhema & Jideani, 2015; Agustini, 2017; Simons, 2018). The parameter redness (a*) were positive values for reddish colours, and the yellowness (b*) also positive values for yellowish colours. The redness was highest for the roasted malt syrup, and the yellowness was lowest for toasted malt syrup, attributed to the differences in the kilning temperatures and time (Pathare *et al.*, 2013). The BGN speciality malts syrups chroma was lowest for the toasted BGN speciality malt syrup. The chroma values refer to the colour saturation, where low chroma values are weak and high chroma values are highly saturated or strong (Jha, 2010; Agustini, 2017). The chroma values for the study on rice syrup were low, with a dark brown colour range compared to this study (Woffenden *et al.*, 2001; Osuji *et al.*, 2020).

The speciality malts hue angle was higher than the syrups due to heat application during mashing. Hue angle (h^{o}) is the attribute of a colour distinguished by the red, yellow, blue, green, or purple object (Agustini, 2017). The way humans perceive colour can be

accurately determined by hue angle, as shown in Figure 5.4 (Osuji *et al.*, 2020). The BGN speciality malt syrup hue angle range of 59.20 to 73.53^{o} indicated reddish-yellow.

a b c d

Figure 5.4: Bambara groundnut speciality malt syrups (a) Base malt syrup, (b) Caramel malt syrup, (c) Roasted malt syrup, (d) Toasted malt syrup

The hue angle (h^{o}) is consistent with other studies that reported a decrease in h^{o} value during heat application to syrup (Pathare *et al.*, 2013; Agustini, 2017). In addition, the colour of the BGN speciality malt syrup indicated that more colour developed during the wort boiling based on temperature and time (O'Rourke, 2002; Willaert & Baron, 2011).

Malt extract boiling generally increases wort colour due to the formation of melanoidins, the caramelization of sugars, and polyphenols' oxidation (Willaert, 2006). The

application of heat reduced the lightness (L*), redness (a*), yellowness (b*) and the hue of the syrup for all BGN speciality malt syrups, while hue angle (h^o) increased. Also, the toasted malt syrup exhibited the darkest colour for the colour parameters (CIE L*a*b*, chroma). The decrease in lightness, redness, yellowness of speciality malt syrup has been attributed to the formation of colour compounds (melanoidins) due to Maillard reaction during kilning and further heating when producing syrups, thus providing desirable colours to food produced with them (Osuji *et al.*, 2020).

The speciality malt, extracts and syrups are good sources for natural colour enhancement in food industries for beverages, baked, and culinary recipes (Giebel, 2015; Felix, 2020). The colour enhancement can be attained using a base malt ratio with specialised malt flours, malt extracts, or syrup (Hansen & Wasdovitch, 2005; Giebel, 2015). The speciality malts and syrups in this study exhibited colours desirable in the food industries, which could be used to impact the colours of baked goods and breakfast meals as the popular barley malt (Carvalho *et al.*, 2016). Furthermore, being significantly different could mean that the speciality malts and syrups could impact different shades of colours as ingredients in product formulation.

5.9.2 The pH characteristics of the Bambara groundnut speciality malts and syrups

The pH for the BGN speciality base (BM) caramel (CM), roasted (RM), and toasted (TM) malts ranged from 6.30 to 6.52. The base, roasted and toasted malts were not significantly different, as shown in Figure 5.5. However, the base, roasted and toasted malt exhibited higher pH than the caramel malt. The caramel malt had the lowest pH value, while the higher pH in the three speciality malts has been attributed to variations in temperature and time (Vandecan *et al.*, 2011). However, the high pH of the base malt is a characteristic of base malt (DeLange, 2013a). During their study, Vandecan *et al.* (2011) showed that the time and temperature of roasting caramel malt resulted in a pH increase. Their results showed that malt pH decreased with increasing kilning temperature due to acidic Maillard reaction products reductones and melanoidins. However, there was a pH increase after the initial decrease with increased roasting temperature to 180°C, similar to this study. The pH increase was ascribed to the decline in the concentration of acidic components due to evaporation, further conversion and polymerization reactions (Coghe *et al.*, 2006; Vandecan *et al.*, 2011). In their study, Geurts (2016) noted that the malt pH depends mainly on the production method used to create speciality malt. The effects of pH on speciality malts have been studied, where it was discovered that dark malts tend to exhibit higher pH than pale (base malt) and light caramel malt (Liscomb *et al.*, 2015; Geurts, 2016). The high pH is due to the dark roasted and toasted malt products being roasted at high temperatures

that are enough to use Maillard reaction, caramelisation, and pyrolysis, which can affect the pH of the speciality malt (Geurts, 2016; Ofoedu *et al.*, 2020)

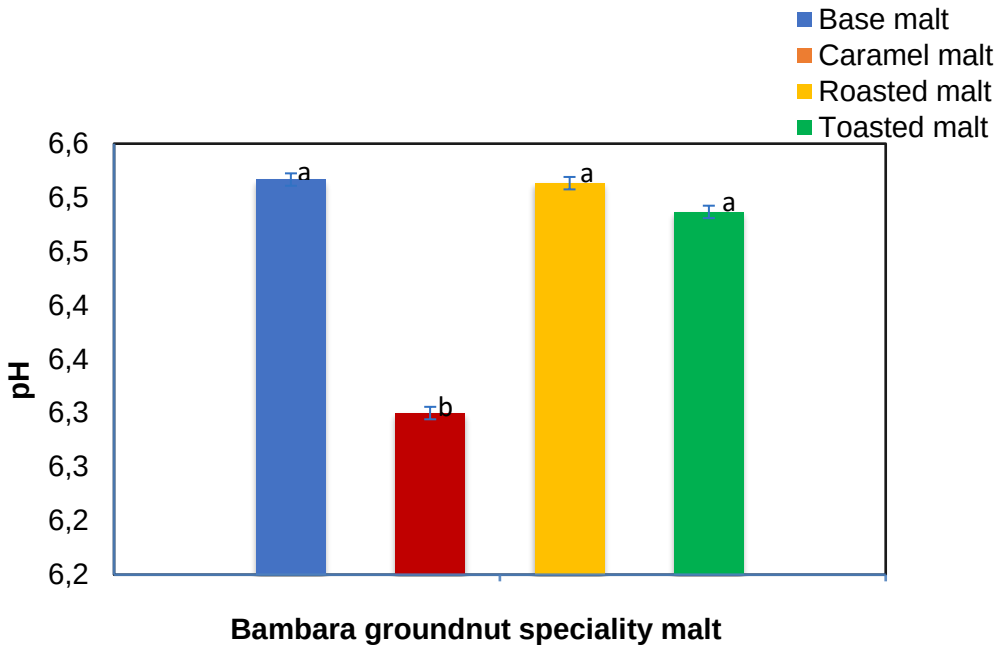

Figure 5.5: pH characteristics of Bambara groundnut speciality malt[1]

[1]Values are mean ± standard deviation of triplicate values, mean values on the bars with different letters are significantly different ($P < 0.05$)

The characteristics pH of the BGN speciality malt syrups for the base, caramel, roasted and toasted malt syrups were 5.52, 5.13, 5.46, 5.71, respectively, in Table 5.3. The pH of the toasted malt syrup was much higher than the base, caramel, and roasted malt syrups, with a significant ($p = 0.000$) difference. pH is crucial in wort production; it regulates the activity of the enzymes (external and internal) in the mash (Cela *et al.*, 2020). The mashing and wort boiling period is the application of heat treatment that can separate the calcium ion (Ca2+) bound with phosphates (K_2PO_4) and polypeptides to form insoluble compounds by the release of hydrogen ion (H+) and decrease of the wort pH (Palmer, 2006; DeLange, 2013b; Cela *et al.*, 2020; Ofoedu *et al.*, 2021). Due to boiling, the wort becomes acidic with a range of 0.1–0.3 pH units for a typical boiling process due to the melanoidins formation (Willaert, 2006; Willaert & Baron, 2011). Also, the pH of the BGN

caramel, roasted and toasted speciality malt syrups, was lower and attributed to the formation of acids from sugars compared to the base malt syrup (Willaert, 2006; DeLange, 2013b; Geurts, 2016).

Table 5.3 pH characteristics of Bambara groundnut speciality malt syrups[1]

Bambara groundnut speciality malt syrup	pH
Base malt	5.52 ± 0.06[a]
Caramel malt	5.13 ± 0.04[b]
Roasted malt	5.46 ± 0.03[a]
Toasted malt	5.71 ± 0.01[c]

[1]Mean values on the column with different superscripts are significantly different ($p \leq 0.05$)

Due to heat application during boiling, the pH is relatively low after malt syrup production (Ofoedu *et al.*, 2021). The pH of the wort produced from chickpea, yellow pea, common vetch, and green lentil were 5.44, 5.7, 5.53 and 5.51, respectively (Gasiński *et al.*, 2021). These are in the same range as this study's BGN speciality malt syrup. The pH of the BGN speciality malt syrups is in the same range as the barley malt syrups understudied in the specific European brewery convention range (Fox, 2009; Stewart, 2017; Gasiński *et al.*, 2021; Gebeyaw, 2021). The BGN speciality malt syrups exhibiting a similar pH range might make them useful in brewing industries as a substitute for malted barley. Thus, producing BGN malt syrups isothermally, as described in this study, gave products that could be used in product formulation, promoting BGN as a functional ingredient.

5.9.3 The protein content of Bambara groundnut speciality malts and syrups

The protein content of the base, caramel, roasted and toasted BGN speciality malts are 15.41, 14.12, 16.22, 17.58, respectively, as shown in Figure 5.6. The protein contents for the BGN speciality malts are significantly ($p = 0.000$) different, with caramel malt having the lowest protein content. The difference in the protein content could be due to different kilning temperatures and times. It was noted that high kilning (range of 40 to 60^{o}C) temperatures of malted HomChaiya rice influenced the protease enzymes similar to this study, which invariably increased the soluble protein and amino acids (Lekjing & Venkatachalam, 2020). Therefore, the increase is attributed to the soluble protein as kiln temperature increased due to an acceleration of proteolytic activities. In contrast to the result of this study Diedericks et al. (2020) reported reduction in protein of BGN seeds subjected to roasting from 70 to 179^{o}C for soaked and unsoaked BGN seeds. Attributing the reduction to the

exposure to high temperature due to denaturation of proteins depending on their thermal stability. However, Skendi & Papageorgiou (2018) recorded no difference in protein content at different temperatures up to 100^{o}C in Greek barley.

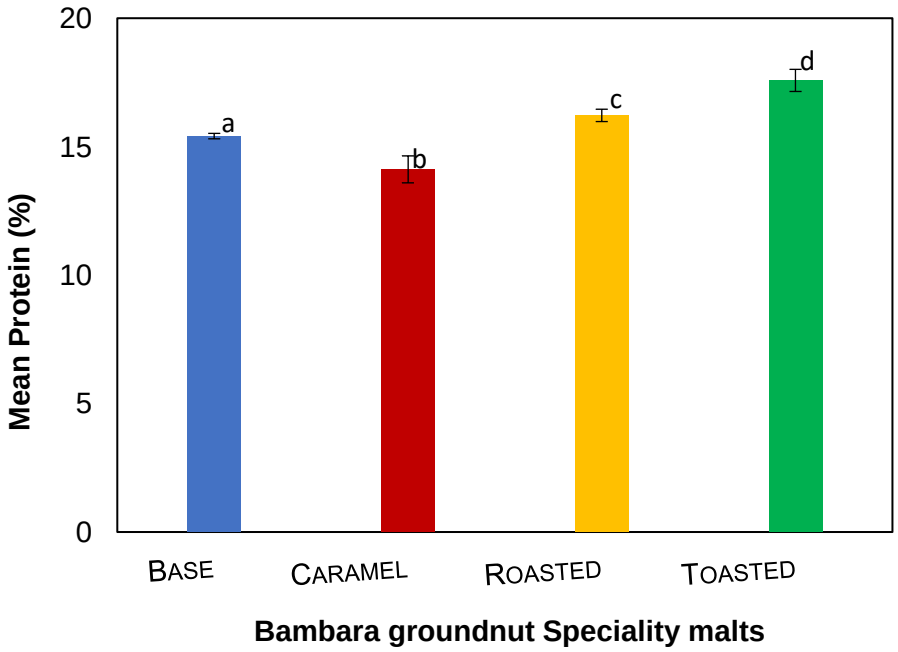

Figure 5.6: protein content of Bambara groundnut speciality malts[1]

[1]Values are mean of triplicates ± standard deviation, mean values on the bars with different letters are significantly different (P < 0.05)

The Bambara groundnut speciality malt syrups protein content was lower after mashing and boiling, ranging from 9.73, 10.37, 11.10 to 11.35 for the base, caramel, roasted and toasted malt speciality syrups, respectively in Figure 5.7. Based on the Kruskal-Wallis test, protein distribution was the same across the syrups, showing no significant difference. The use of legume malts to produce gluten-free worts and malt extracts with high protein content with a reduced amount of anti-nutritional constituents and increased antioxidants has been documented (Black et al., 2018; Iannetta et al., 2018; Gasiński et al., 2021; Ofoedu et al., 2021). Wort boiling is a thermal process whereby various chemical, physicochemical, physical, and biochemical reactions occur. The boiling of wort is important for sterilising wort, stopping enzymatic reactions, water evaporation from the wort,

unwanted aroma compounds removal, and hot break or hot trub, which is the precipitation of wort protein contents insoluble coagulum (Willaert, 2006; Kü *et al.*, 2007).

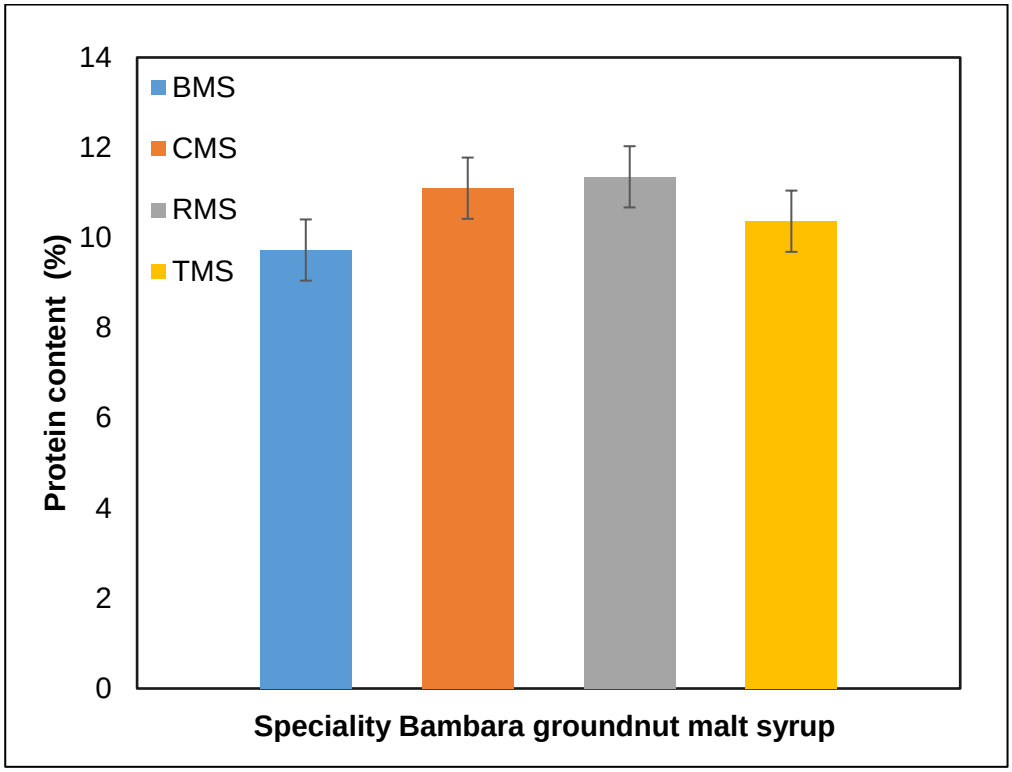

Figure 5.7: Protein content of the BGN speciality malt syrup, BMS- Base malt syrup, CMS- Caramel malt syrup, RMS- Roasted malt syrup, TMS- Toasted malt syrup

The protein content of malt is dependent on the enzyme-to-substrate ratio, that is, the ratios of α- and β-amylases/starch and endo-peptidases/proteins (Willaert, 2006). The protein contents of the malt extract decrease after boiling, which matches the results reported by Bei *et al.* (2009) and Osman *et al.* (2003). The reduction in protein can be attributed to protein degradation during mashing and wort boiling (Parkes, 2002; Ferreira *et al.*, 2018). In contrast to the reduction in protein observed in this study and literature, Osman *et al.* (2003) established that the barley wort protein content increased and ascribed to the elevated stability of the soluble proteins. However, the BGN speciality malt syrup exhibited a good proportion of protein content that could benefit consumers.

5.9.4 Amylase activities of Bambara groundnut speciality malts and syrups

The α and β-amylase activities for the base, caramel, roasted and toasted BGN speciality malt were 1.01, 0.21, 0.29, 0.15 CU/g and 0.11, 0.10, 0.10, 0.06 BU/g, respectively in Table 5.4. The amylase activities of the BGN speciality malts differed significantly (p = 0.000) across the malt types. Studies have shown that amylase activities change with change in kilning temperature and time which is similar to the results from this study (Karababa *et al.*, 1993; Uvere *et al.*, 2000; Gebremariam *et al.*, 2013; Guido & Moreira, 2013; Lekjing & Venkatachalam, 2020). Kilning temperature and time of germinated sorghum grains reduced the α- and β- amylase activities (Uvere *et al.*, 2000). Uriyo (2001) observed that kilning black-eyed pea at higher temperatures reduced the α-amylase activities, and β-amylase activity could not be detected in the germinated cowpea. The α and β- amylase of cowpea, buckwheat, sorghum, teff and barley malt were found to decrease linearly with an increase in drying temperature (Evans *et al.*, 1997; Patricia *et al.*, 2005; Gebremariam *et al.*, 2013; Abuajah, 2017; Yousif & Evans, 2020). As with other studies on sorghum, buckwheat, teff, barley and cowpea, β-amylase was low or absent after kilning, which correlates with the findings on BGN speciality malt (Uriyo, 2001; Abuajah, 2017). In addition, the α and β- amylase decreased with change in kilning temperature and time where the β-amylase showed the lowest value. The resultant reduction in α and β-amylase regarding kilning temperature and time was because diastatic enzymes can only survive in mild kilning due to the formation of heat-stable complexes in the starch granules (Bathgate, 2016).

Table 5.4 Bambara groundnut speciality malts α and β- amylase activities[1]

BGN speciality malt	Alpha-amylase	Beta-amylase
Base	1.01 ± 0.01^{a}	0.11 ± 0.00^{a}
Caramel	0.21 ± 0.00^{b}	0.10 ± 0.00^{b}
Roasted	0.29 ± 0.00^{c}	0.10 ± 0.00^{c}
Toasted	0.15 ± 0.00^{d}	0.06 ± 0.00^{d}

[1]Values are mean of triplicates ± standard deviation, values in the same column with different superscripts are significantly different (p ≤0.05)

The decrease in the enzymatic activity could thus be due to the heat denaturation of grains, known as the enzyme inactivating phase (Karababa *et al.*, 1993; Evans *et al.*, 1997; Ferrari-John *et al.*, 2017). The barley α- amylase is more thermostable than β- amylase; the α and β-amylase of the BGN speciality malts have shown similar thermostability (Patricia *et al.*,

2005; Gebremariam *et al.*, 2013). There was an increased inactivation by kilning due to denaturation by heat application (Rani, 2012; Gebremariam *et al.*, 2013; Ferrari-John *et al.*, 2017). Despite the heat application during kilning, mashing and boiling of malt wort to produce syrup, some amylase survives (Lewis & Young, 2001a; Gupta *et al.*, 2010; Bathgate, 2016; Poisson *et al.*, 2020).

The base, caramel, roasted, and toasted BGN speciality malt syrups (BMS, CMS, RMS, and TMS) α- amylase were 0.39, 0.31, 0.30, 0.31 CU/g and β-amylase are 0.14, 0.13, 0.15, 0.21 BU/g respectively as shown in Table 5.5. There is a significant ($p = 0.000$) difference across the amylase activities of the speciality malt syrups. The increase in the α and β-amylase activities observed in the BGN speciality malts syrups after wort boiling is due to the activities of the enzymes (Willaert & Baron, 2011; Parés Viader *et al.*, 2021). The production of malt-based syrups involves producing the malt, mashing process to produce wort from the malt and concentration of the wort to malt syrup by boiling (Gholami Aghel *et al.*, 2016; Gasiński *et al.*, 2021). Characteristics of malt syrup are brown, sweet, gluey liquids with diastatic enzymes (base malt) or without diastatic enzymes (speciality malt) (Lewis & Young, 2001b). Speciality malts are very important for enhancing and improving malts wort (syrup) by improving their colour and flavour (Lewis & Young, 2001b).

Table 5.5 Amylase activities of Bambara groundnut speciality malt syrups[1]

BGN speciality malt syrup	α-amylase	β-amylase
Base malt	0.39 ± 0.00^{a}	0.14 ± 0.00^{a}
Caramel malt	0.31 ± 0.00^{b}	0.13 ± 0.00^{b}
Roasted malt	0.30 ± 0.00^{b}	0.15 ± 0.00^{c}
Toasted malt	0.31 ± 0.00^{c}	0.21 ± 0.00^{d}

[1]Values are mean of triplicates ± standard deviation, values in the same column differ significantly ($p \leq 0.05$).

The α-amylase activity of the base malt syrup was the highest while the roasted malt syrup showed to be the lowest. The β-amylase is thermally unstable; it is denatured at high temperatures, thus the low content in this study (Lewis & Young, 2001a). The mashing and wort boiling temperature could have affected the β-amylase content due to the mashing temperature of 60°C (Parés Viader *et al.*, 2021). In their study, De Schepper *et al.* (2021) noted that α-amylase and β-amylase are temperature-dependent. α-amylase is inactivated at temperatures 63–71°C and β-amylase at 54–66°C (De Schepper *et al.*, 2021). These two

enzymes are very important as α-amylase breaks complex, insoluble starch molecules into smaller, soluble molecules, which is more stable thermally. α-amylase produces low molecular weight sugars, glucose, maltose and maltotriose. β-amylase being an unstable enzyme at high temperatures, produces only maltose. Once its activity reaches a peak, it declines and then drops at an increase in temperature (O'rourke, 2002; German brewing, 2016; Mosher & Trantham, 2017). The activities of these enzymes (α- and β-amylase) are relatively dependent on the temperature and time of mashing, and wort boiling (De Schepper *et al.*, 2021), as shown in this study. The inactivation is thus attributed to the starch hydrolyses by the two enzymes. α-amylase is an endo-acting enzyme that degrades starch during mashing, cleaving α- 1,4-D-glucosidic linkages to produce oligosaccharides and limit dextrins (Lewis *et al.*, 2001; Henson *et al.*, 2018; Rani & Bhardwaj, 2021). On the other hand, β-amylase is an exo-acting enzyme, hydrolysing starch and oligosaccharide α-1,4-D-glucosidic linkages from the non-reducing end to produce maltose (Montanari *et al.*, 2005; Chandrasekaran, 2015; Herrera-Gamboa *et al.*, 2018). Thus, having enzyme-rich malt and syrup would greatly depend on the extraction temperature due to the heat-sensitive nature of the α- and β-amylase amylases. However, boiling the BGN syrup at temperatures lower than 60^{o}C could increase amylase concentrations.

5.9.5 Total polyphenols content and antioxidant activities of Bambara groundnut speciality malts and syrups

Total polyphenols content and antioxidant activities of BGN speciality malts are illustrated in Table 5.6. There was a significant ($p = 0.000$) difference with an increase in total polyphenols and antioxidants content from 1.50 to 3.11 mg GAE/g. Also, the antioxidants increased, where FRAP ranged from 4.89 to 15.89 µmol AAE/g and DPPH ranged from 6.36 to 14.13 µmol TE/g. The increase in total polyphenols and antioxidants during kilning may be attributed to the extraction and release of bound phenolic compounds ((+)-catechin and ferulic acid) due to friable tissue created by kilning (Lemmens *et al.*, 2019; Gąsior *et al.*, 2020; Ambra *et al.*, 2021). This friable tissue made it easy to extract the phenolic compounds better by synthesising some hydrolytic enzymes in studied grains like barley, quinoa, millet, and sorghum (Lu *et al.*, 2007; Dvořáková *et al.*, 2008; Inns *et al.*, 2011; Carvalho *et al.*, 2014; Jannat, 2015; Carciochi *et al.*, 2016; Nemzer *et al.*, 2019).

The total polyphenols by Folin–Ciocâlteu reagent (FCR), antioxidant activities by ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay showed an increase with the increase in kilning time and temperature. Increased antioxidant properties are contributed by the Maillard reaction products (MRPs) produced during kilning of the malting process (Sharma et al., 2020). Mainly the roasting processes

exhibit heat-induced antioxidants MRP called melanoidins (Coghe et al., 2004c; Sovrano et al., 2006). Continuous research on antioxidants during the malting process, especially the kilning time and temperature, has clearly shown that dark speciality malt had the most significant antioxidant activities (Coghe et al., 2004c; Pejin et al., 2009).

Table 5.6 Total polyphenols and antioxidant activities of Bambara groundnut speciality malts[1]

Bambara groundnut speciality malt	Polyphenol (mg GAE/g)	FRAP (µmol AAE/g)	DPPH (µmol TE/g)
Base	1.50 ± 0.09[a]	4.89 ± 0.30[a]	6.36 ± 0.05[a]
Caramel	1.55 ± 0.07[a]	5.86 ± 0.23[a]	6.81 ± 0.92[a]
Roasted	3.11 ± 0.25[b]	15.39 ± 0.56[b]	14.13 ± 0.13[b]
Toasted	2.86 ± 0.23[b]	15.89 ± 0.90[b]	13.70 ± 1.22[b]

[1]Values are mean of triplicates ± standard deviation, Values in the same column differ significantly ($p \leq 0.05$). GAE-Gallic acid equivalent, AAE-ascorbic acid equivalents TE-Trolox equivalent

The BGN speciality malt syrups exhibited total polyphenols of 0.72, 0.65, 1.20 and 1.60 mg GAE/g, FRAP 2.00, 1.20, 2.42 and 4.43 µmol AAE/g and 1.56, 1.51, 2.11 and 2.96 µmol TE/g, for base, caramel, roasted and toasted BGN speciality malt syrups respectively in Table 5.7. There was a significant difference across the BGN speciality syrups. The total polyphenols activity in the toasted malt syrup was the highest, while the caramel malt had the lowest value.

Table 5.7 Total polyphenols and antioxidant activities of Bambara speciality malt syrups[1]

BGN speciality malt syrup	Total polyphenols (mg GAE/g)	FRAP (µmol AAE/g)	DPPH (µmol TE/g)
Base malt	0.72 ±0.04[a]	2.00 ± 0.14[b]	1.56 ± 0.13[a]
Caramel malt	0.65 ± 0.03[a]	1.20 ± 0.02[a]	1.51 ± 0.13[ab]
Roasted malt	1.20 ± 0.05[b]	2.42 ± 0.05[c]	2.11 ± 0.30[b]
Toasted malt	1.60 ± 0.19[c]	4.43 ± 0.18[d]	2.96 ± 0.49[c]

[1]Values are mean of triplicates ± standard deviation, values in the same column differ significantly ($p \leq 0.05$). GAE-Gallic acid equivalent, AAE-ascorbic acid equivalents TE-Trolox equivalent

Since Maillard reaction activities enhance the colour of the speciality malt during kilning of malt and boiling of wort, there is an increase in total polyphenols after boiling the malts extracts to produce syrup (Coghe *et al.*, 2004c). Coghe *et al.* (2004c) showed in their investigation that dark speciality malts and their extracts had the highest antioxidant activities due to higher heat application, as heat treatment is linked with an increase in antioxidant activity. The antioxidant activity of speciality malt wort increase was attributed to redox-reducing antioxidants developed during curing and roasting, giving rise to malt colour change and antiradical antioxidant activity formed during the Maillard reaction (Woffenden *et al.*, 2002a; Carvalho *et al.*, 2016). Samaras *et al.* (2005) noted that the antioxidant activity of phenolic compounds and antioxidants were higher for the darkly kilned malts as Maillard reaction products increased. Maillard reaction products have antioxidant properties that influence the oxidative stability of wort (Coghe *et al.*, 2004c, 2006; Carvalho *et al.*, 2016). However, studies have shown that malt kilned at high temperatures have the most increased antioxidant activity contributing to higher intensities of Maillard reaction products (Inns *et al.*, 2011; Guido & Moreira, 2013). Congress worts produced from vetch, green lentil, chickpea, and yellow pea malts had high phenolic and antioxidant components (Gasiński *et al.*, 2021). The Folin–Ciocalteu, DPPH and FRAP assays vetch had the highest total polyphenols and antioxidants (Gasiński *et al.*, 2021). The high content of total polyphenols and antioxidants is attributed to the dark colour and hardcover characteristics of this type of legume seeds having higher flavonoids and condensed tannins, which may increase antioxidant activity (Altieri & Nicholls, 2012; Gasiński *et al.*, 2021). Research works, and reports have noted that legumes with dark coloured and tough seed coats have strong antioxidant characteristics (Segev *et al.*, 2010; Altieri & Nicholls, 2012; Jideani & Diedericks, 2014; López-Cortez *et al.*, 2016; Singh *et al.*, 2017). BGN is characterised by tough and coloured (black, dark brown, red, white, and speckled) varieties that could be attributed to the increased antioxidant in BGN speciality toasted malt activities in this study (Jideani & Diedericks, 2014; Mandizvo & Odindo, 2019). Thus, a desirable high total polyphenolic and antioxidant food product could be produced from the BGN toasted malt and syrup.

5.10 Total soluble solid of Bambara groundnut malt syrups

The degree Brix (^{o}Brix) of the BGN speciality malt syrups was 11.57, 9.97, 25.90 and 15.93 ^{o}Brix, as illustrated in Figure 5.8. The ^{o}Brix for roasted malt syrup was the highest, indicating the highest total soluble solids content. A degree Brix (^{o}Brix) is a gram of sucrose in 100

grams of solution. The soluble solids recorded in the legume malt worts by Gasiński *et al.* (2021) without the addition of enzyme consisting of vetch, green lentil, chickpea and yellow pea (2.40, 1.59, 2.39 and 2.80 Plato^{o} (≈ ^{o}Brix)) were lower than the values for BGN malt syrups. Meanwhile, in the malted and un-malted rice syrups production by Ofoedu et al. (2020), the ^{o}Brix was higher for the malted rice syrups peaking at 72.10 °Brix. The high ^{o}Brix value was attributed to increased hydrolytic activity during germination and mashing by releasing more hydrolysates.

Furthermore, it was recognized that the physicochemical characteristics and quality of malts depend on the kilning duration and intensity, which will affect the mashing and wort quality (Skendi & Papageorgiou, 2018). The quality of the extract and malt extract syrup will add value to the production of foods by serving as a source of sweetener, flavour, colour, and enzymes (Baranwal, 2017; Krstanović *et al.*, 2020). The high total soluble content of the roasted malt syrup could be desirable in producing a non-alcoholic beverage that will add natural sweetness to the product and benefit consumers' wellbeing.

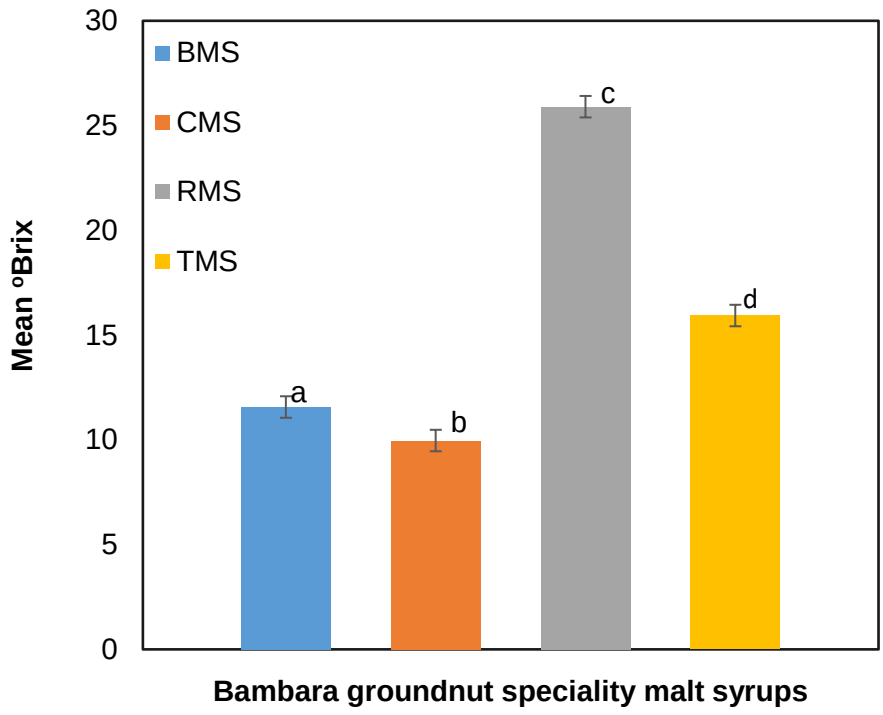

Figure 5.8: Degree Brix of Bambara speciality malt syrups[1]

[1]Values are mean of triplicates ± standard deviation, mean values on the bars with different letters are significantly different (P < 0.05). BMS–Base malt syrup, CMS–Caramel malt syrup, RMS–Roasted malt syrup, TMS–Toasted malt syrup

5.11 Metabolites of the Bambara groundnut speciality Malts

The BGN speciality malts (base malt, caramel malt, roasted malt, and toasted malt) were profiled for metabolites, including the amino acids, sugars, sugar alcohol and organic acids, fatty acids methyl esters (FAME), and volatiles as illustrated in the following sections.

5.11.1 Amino acid compositions of Bambara groundnut speciality malts

The amino acid of the BGN speciality malts was significantly (p = 0.000) different from the base, caramel, roasted to toasted except for leucine which was not significantly different across the BGN speciality malts, as shown in Table 5.8. The non-essential amino acids consist of aspartic acid, glutamic acid, cysteine, serine, proline, alanine, glycine, and tyrosine. Lysine was the highest amino acid for the base, caramel, and roasted malts at 61.97, 52.67 and 38.89 mg/g, respectively, while aspartic acid was the highest for toasted malt at 14.46 mg/g. On the other hand, methionine was the lowest amino acid for all BGN speciality malt types. This is because methionine, a sulphur-containing essential amino acid, is more deficient in legumes than other essential amino acids while rich in lysine (Tiwari *et al.*, 2011; Zhou *et al.*, 2013; Limwiwattana *et al.*, 2016). However, raw BGN has a considerably high amount of methionine ranging from (1.30 to 2.90 g/100g) compared to other legumes (Mazahib *et al.*, 2013; Hardy & Jideani, 2016; Jideani, 2016; Duodu & Apea-Bah, 2017; Sarkar & Sensarma, 2019).

The amino acid profile for the BGN speciality malt (BM, CM, RM and TM) showed higher amino acid contents than the raw BGN seeds. Nzelu (2016) and Chinma *et al.* (2021) noted that germination increases the amino acid content of BGN due to protease activity. However, there was a consistent decline of the amino acids of the BGN speciality malts. The decline has been attributed to different kilning temperatures and the initiation of Maillard reactions between reducing sugars and amino compounds in barley malts (Nie *et al.*, 2010). Samaras *et al.*(2005) noted that the concentrations of amino acids decreased with increased heat treatment applied to barley grains in the production of speciality malts. The decrease in amino acids was also attributed to the Maillard reaction level and sugar caramelisation by Strecker degradation at higher temperatures (Coghe *et al.*, 2004a; Parr *et al.*, 2021). This study showed that BGN speciality malts varied in amino acid concentration due to the drying conditions; hence, the base malt with the highest amino

acid concentration could be optimised for production to use as functional ingredients in food and beverage production.

Table 5.8 Amino acids concentrations of Bambara groundnut speciality malts[1]

	Amino acids concentration (mg/g)			
Essential amino acid	**Base malt**	**Caramel malt**	**Roasted malt**	**Toasted malt**
Lysine	61.97 ± 1.17^{a}	52.67 ± 0.17^{b}	38.89 ± 0.40^{c}	10.72 ± 0.82^{d}
Threonine	15.385 ± 0.05^{a}	12.93 ± 0.08^{b}	13.55 ± 0.11^{c}	9.90 ± 0.01^{d}
Phenylalanine	13.99 ± 0.15^{a}	11.25 ± 0.01^{b}	12.81 ± 0.18^{c}	9.16 ± 0.51^{d}
Valine	12.47 ± 0.31^{a}	11.95 ± 0.06^{b}	12.14 ± 0.01^{ab}	7.91 ± 0.08^{c}
Leucine	11.91 ± 0.23^{a}	10.41 ± 0.10^{a}	10.45 ± 0.54^{a}	11.26 ± 1.42^{a}
Isoleucine	10.60 ± 0.08^{a}	9.97 ± 0.02^{b}	8.46 ± 0.01^{c}	7.25 ± 0.10^{d}
Methionine	4.52 ± 0.04^{a}	4.38 ± 0.04^{b}	4.24 ± 0.06^{c}	1.92 ± 0.05^{d}
Non-essential amino acid				
Aspartic acid	27.45 ± 0.22^{a}	21.84 ± 0.05^{b}	23.00 ± 0.07^{c}	14.46 ± 0.25^{d}
Glutamic acid	22.24 ± 0.06^{a}	19.98 ± 0.01^{b}	21.44 ± 0.35^{c}	13.66 ± 0.04^{d}
Cysteine	22.34 ± 0.01^{a}	12.94 ± 0.25^{b}	15.38 ± 0.12^{c}	7.23 ± 0.36^{d}
Serine	13.10 ± 0.06^{a}	10.51 ± 0.00^{b}	11.59 ± 0.16^{c}	11.75 ± 0.06^{c}
Proline	13.14 ± 0.10^{a}	11.62 ± 0.13^{b}	11.49 ± 0.52^{b}	7.16 ± 0.14^{c}
Alanine	7.87 ± 0.13^{a}	7.35 ± 0.01^{b}	7.40 ± 0.23^{b}	4.05 ± 0.14^{c}
Glycine	7.42 ± 0.01^{a}	6.81 ± 0.03^{b}	5.73 ± 0.03^{c}	2.71 ± 1.93^{d}
Tyrosine	4.73 ± 0.02^{a}	4.26 ± 0.04^{b}	4.02 ± 0.06^{c}	3.06 ± 0.02^{d}

[1]Values are mean of duplicates ± standard deviation, values in the same row differ significantly ($p \leq 0.05$).

5.11.2 Acids, sugars, and sugar alcohol of Bambara groundnut speciality malts

Lactic acid, a non-volatile organic acid, was present in all the BGN speciality malts where toasted malt (0.06 mg/g) had the highest content. There was a significant ($p = 0.000$)

difference in the concentration of lactic acid for the base, caramel, roasted to toasted BGN speciality malts. The higher lactic acid contents have been attributed to the kilning time and temperature by Xiang *et al.*(2006). Comparing two malting regimes of barley, South (1996) noted that kilning time is important for final lactic acids levels in malts where long kilning times lead to high levels of lactic acids. It was suggested that lactic acid must have been produced by dividing the grain microbes of the malt during kilning. The concentrations of the acid, sugar and sugar alcohol in the BGN speciality malts are illustrated in Table 5.9.

Table 5.9 Acid, sugars, and sugar alcohol concentration of speciality Bambara groundnut malts[1]

Acid, sugar, and sugar alcohol (mg/g)	**Bambara groundnut speciality malts**			
	Base	**Caramel**	**Roasted**	**Toasted**
Lactic acid	0.04 ± 0.00^{ab}	0.01 ± 0.00^{a}	0.03 ± 0.00^{ab}	0.06 ± 0.00^{b}
Fructose	0.02 ± 0.00^{a}	0.07 ± 0.00^{a}	0.34 ± 0.03^{b}	0.02 ± 0.00^{a}
Sucrose	4.77 ± 1.10^{a}	5.27 ± 1.50^{a}	9.08 ± 3.10^{b}	6.33 ± 0.70^{ab}
Myo-inositol	0.04 ± 0.00^{a}	0.22 ± 0.10^{ab}	0.47 ± 0.10^{bc}	0.76 ± 0.40^{c}

[1]Values are mean of duplicates ± standard deviation, values in the same row differ significantly ($p \leq 0.05$)

Sugars and sugar alcohols consisting of fructose, sucrose and myo-inositol were also present in the BGN speciality malts in appreciable concentrations, as are illustrated in Table 5.10. The toasted malt (0.76 mg/g) had the highest concentration for myo-inositol, while the roasted had a higher concentration of fructose and sucrose at 0.34 and 9.08 mg/g, respectively. However, the fructose and sucrose concentrations for the BGN speciality malts were not significantly different. The varying concentration of sugars in the BGN speciality malt was attributed to the intensity and duration of the heat applied during kilning, resulting in Maillard reaction formation and sugar caramelisation common in extremely roasted malts (Samaras et al., 2005). However, Almeida et al. (2014) noted that sucrose is more abundant in the pilsner malt (base malt variety) profiled by high performance-liquid chromatography (HPLC). However, it was suggested that the heat application during kilning increased the sugar composition of the final malt product as sugar was used as precursors for the thermally generated compounds (Lekjing & Venkatachalam, 2020). Therefore, based on the sugar, sugar, alcohol, and acid concentration of the BGN speciality malt in this study, toasted malt could be produced for its use in the production of various food products, particularly in beverage industries.

5.11.3 Fatty acids methyl esters (FAME) of Bambara groundnut speciality malts

The FAME identified in the base, caramel, roasted and toasted Bambara groundnut speciality malts were palmitic, oleic, linolelaidic, linoleic and arachidic acid, as illustrated in Figure 5.9. The metabolite levels on the heatmap correspond to the colour temperature, and higher temperatures indicated higher levels of FAME compounds. The BGN speciality malts exhibited FAME in different concentrations. Linoleic acid was abundant in all the BGN speciality malt types, while oleic acid was the lowest and was absent in the roasted malt.

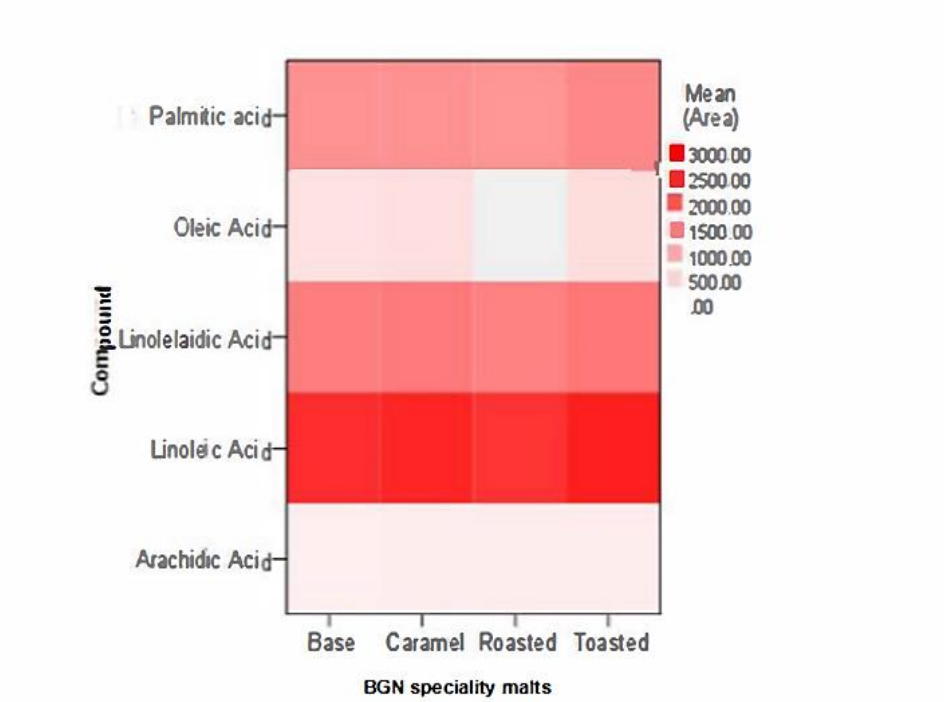

Figure 5.9: Heat plots of saturated, mono-saturated, and polyunsaturated FAME of speciality Bambara groundnut malts

The major fatty acid components in raw BGN are caprylic, capric, lauric, palmitic, palmitoleic, oleic and linoleic acids (Okonkwo & Opara, 2010; Ibrahin & Ogunwusi, 2016). Whereby linoleic acid was found to be the highest fatty acid in raw BGN seeds (Adeleke *et al.*, 2018), which could be contributing to its concentration in the speciality malts. Similar to this study, Özcan *et al.* (2018) reported that linoleic acid content in barley malt increased during the malting process (steeping, sprouting and drying), whereas oleic and palmitic acid

content decreased. Bravi *et al.* (2012) also noted that the linoleic acid increased in barley malt after kilning, which could be why the BGN speciality malt in this study exhibited high concentration. An increase in heat application to linoleic acid has been found to increase its concentration which was attributed to varying lipids biosynthesis during the malting process (Gerčar & Šmidovnik, 2002; Mateos *et al.*, 2010; Bravi *et al.,* 2012; Farinon *et al.*, 2022). Linoleic acid, oleic acid and palmitoleic acids are essential unsaturated fatty acids necessary in human food to prevent certain heart diseases (Okonkwo & Opara, 2010; Bagchi *et al.*, 2015; Boye, 2015; Halimi *et al.*, 2020; Tomislav, 2021). Being abundant in the BGN speciality malt across all products could benefit human health and encourage its production in large quantities.

5.11.4 Volatile metabolites in Bambara groundnut speciality malts

A total of 29 volatile metabolites were identified in the BGN speciality malts based on retention times and mass spectrometric data from MS libraries by HS-GC-FID. The volatile compounds consisted of pyrazine, furans, aldehydes, ketones, esters, and alcohols. The most abundant volatile compound in the BGN speciality malts was the pyrazine, 2,5-dimethyl, higher in the toasted malt. Conversely, the lowest volatile compound was the 2,3,5-Trimethyl-6-ethylpyrazine in all the BGN speciality malts. The volatile compounds in the speciality malts are on the heatmap illustrated in Figure 5.10.

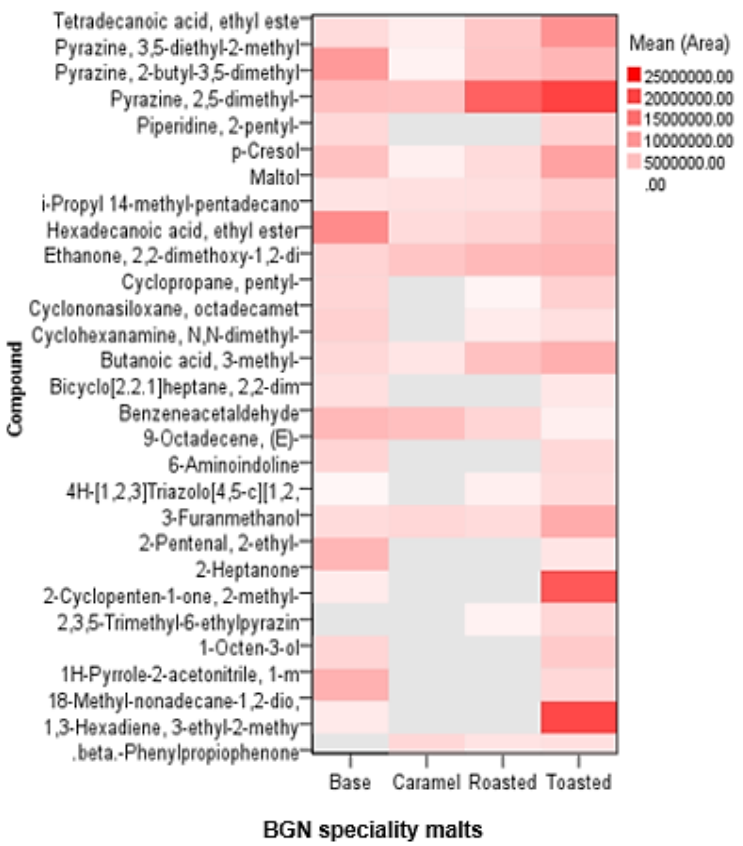

Figure 5.10: Heat plots of the volatile metabolites of Bambara groundnut speciality malts

The most abundant volatile in the BGN speciality malts were the pyrazines. Pyrazines are volatile compounds with monocyclic aromatic rings with two nitrogen atoms. Foods can contain different groups of pyrazines, which consist of alkyl, methoxy and sulphur containing chains (García-Lomillo & González-SanJosé, 2018). Pyrazine, 2,5-dimethyl is, however, the most abundant in the BGN speciality malts. It is characterised by chocolate and roasted nuts flavours (Hui *et al.*, 2007). Thus, it is an essential flavour compound in roasted food products, especially roasted coffee (Farah, 2009). In addition, it is used as a flavour additive and odorant in foods such as cereals; it also occurs naturally in asparagus, green tea, crispbread, malt, raw shrimp, soya, and wheat bread (Maga & Sizer, 1973; Wang *et al.*, 2021; Yan *et al.*, 2021; Yu *et al.*, 2021). Its high concentration in the BGN toasted malt could be attributed to the subjection to higher temperatures after initial drying of 50^{o}C. Methylpyrazine volatile compounds, heterocyclic volatiles, are formed by the Maillard reaction and are also common with the pyrolysis process at higher

temperatures and very low moisture contents (Channell *et al.*, 2010; Parr *et al.*, 2021). The pyrolysis process thus suggested that the maltol present in the BGN speciality malts was formed in addition to Maillard reactions which account for its higher concentration in toasted malt due to its low moisture and high-temperature drying.

Maltol (3-Hydroxy-2-methyl-4-pyrone), a naturally occurring organic compound used as a flavour enhancer, is found only in highly roasted speciality malt like roasted and toasted malts (Oliver, 2012). Maltol is formed due to the Maillard reaction and is characterised by a sweet baked aroma typical in highly heated malts (Parr *et al.*, 2021). The impact of different times and temperatures applied during the caramelisation process of roasted and toasted malt developed the caramel-like flavour maltol (Vandecan *et al.*, 2011). Maltol is a safe, reliable, natural antioxidant, food preservative and flavour (Mi *et al.*, 2018). It is found in baked products, red ginseng root, coffee, chicory, soybeans, bread crusts, and caramelised foods (Zhang *et al.*, 2012; Rögner *et al.*, 2021). It has also been used in catalysis, cosmetics, pharmaceutical formulation, and food chemistry (Anwar-Mohamed & El-Kadi, 2007; Krishnakumar *et al.*, 2014). In addition, it can be used to treat anaemia, tumour, nerve cell oxidative stress and kidney damages (Song *et al.*, 2015; Mi *et al.*, 2018). Studies have also shown that maltol reduced acute alcohol-induced liver injury, prevented oxidative injury through activating some signalling pathways and prevented cisplatin-induced acute kidney injury (Han *et al.*, 2015; Li *et al.*, 2015).

The lowest volatile compound was the 2,3,5-Trimethyl-6-ethylpyrazine, mainly in roasted malt. It is a nitrogen-containing compound in the pyrazines group of volatile heterocyclic (Yu *et al.*, 2021). It is characterised by an earthy, nutty, roasty flavour formed during roasting at high temperatures between 135 and 250^{o}C (García-Lomillo & González-SanJosé, 2018). It is also a chocolate enhancer used in foods containing coffee, cocoa, meat and potatoes as a roasted flavour (Mortzfeld *et al.*, 2020).

The volatiles in the BGN speciality malts has flavour characteristics used in the food industries to enhance and improve consumers' acceptability of food products (García-Lomillo & González-SanJosé, 2018; Mortzfeld *et al.*, 2020). These days organic and natural labels have been gaining popularity as consumers become more aware of the ingredients in their food. Due to the high demand of consumers to eat organically grown food, the need for volatile flavours has increased, and there is a need to extract these volatiles from natural products for use in food production (Fan & Qian, 2006; Mortzfeld *et al.*, 2020; Ismarti *et al.*, 2021). Thus, toasted malt with more abundant volatiles like maltol and pyrazine, 2,5-dimethyl could be used for food and beverage production. The physicochemical and biochemical characteristics of the speciality BGN malts and their syrups produced from

optimum amylase malt showed good characteristics that can be incorporated into food production as ingredients or condiments.

5.12 Conclusions

This study successfully produced speciality Bambara groundnut malts and their corresponding syrups from the amylase rich green BGN malts steeped at 36 h and sprouted at 96 h. The speciality malts and syrups exhibited colours desirable in the food industries, which could be used to impact different shades of colours as ingredients in product formulation in baked goods. The BGN speciality malt syrups exhibited a similar pH range to malted barley syrup, making it a functional ingredient in the beverage industries. Bambara groundnut speciality roasted malt and toasted malt syrup exhibited favourable protein concentration compared to base and caramel malts, which could benefit human health when consumed. The enzyme activities were affected by heat application during malt kilning and extract boiling due to the heat-sensitive nature of the α- and β-amylase amylases. However, boiling at temperatures lower than 60^{o}C could be recommended for the production of BGN syrups with higher amylase concentrations. The toasted malt and its syrup exhibited the highest total polyphenolic and antioxidant activities, which could make it a desirable functional food product ingredient. The ^{o}Brix of the roasted malt syrup was the highest, which could be a desirable attribute in producing non-alcoholic beverages by adding natural sweetness to the product and being beneficial to consumers wellbeing. The profile of metabolite components in the speciality BGN malt included amino acids, fatty acid methyl esters, sugars, sugar alcohol and acid and volatiles. These compounds identified in the BGN speciality malt could add value to the sensory properties, nutritional and functional benefits of BGN. Thus, the speciality Bambara groundnut malt possesses components that can be beneficial and incorporated into human diets for their health benefits.

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CHAPTER SIX
GENERAL SUMMARY AND CONCLUSIONS

This study aimed to profile the metabolites of Bambara groundnut malting process and the characteristics of its malt extracts. The objective of this study was to:

1. Establish the malted BGN seeds with optimum amylase enzyme activities.
2. Determine the effect of the malting process on BGN metabolites.
3. Establish the physicochemical and biochemical characteristics of BGN speciality malt and syrups obtained from the optimum BGN malt.

The first objective of chapter three was to establish the enzyme and antioxidant activities of malted Bambara groundnut as affected by steeping and sprouting times. The objective was achieved by steeping at 25 $\pm$ 3^{o}C for 36 and 48 h and sprouting was carried out up to 144 h at 30^{o}C. The α- and β-amylase activities of the sprouted samples were analysed by the enzymatic Ceralpha Method (K-CERA, Megazyme) kit and enzymatic Beta-Amylase (Megazyme, K-BETA3) kit, respectively. Optimum α-and β-amylases were established by steeping at 36 h and sprouting at 96 h. Thus, establishing the hypothesis that the malting process will affect the amylase enzyme activity of BGN seeds.

The second objective was achieved in chapter 4, where the effect of steeping and sprouting times on the metabolites of malted Bambara groundnut was established. Metabolite profiling method based on capillary gas chromatography-mass spectrometry (GC-MS), gas chromatography with flame ionisation detection (GC-FID) and Headspace Gas Chromatography-Flame Ionization Detector (HS-GC-FID) was employed to study the time-dependent metabolic changes in the course of the malting process. The objective was established by identifying and quantifying the metabolite components based on the steeping and sprouting times applied to the BGN seeds. These metabolites consisted of the Fatty Acid Methyl Esters (FAME) and hydrocarbon, sugars, acids, sugar alcohols, and amino acids. There was a reduction in fatty acid methyl esters (FAME) with increased soaking and sprouting times. The acids, sugar and sugar alcohol of the malted BGN seeds were also reduced in the course of malting BGN seeds. However, the amino acid concentration improved during the cause of sprouting. A total of 62 volatile metabolites was detected in the raw, steeped, and sprouted BGN seeds. The abundant volatile composition showed that the major contributors to the time-dependent dynamic were the hydrocarbons, organic, alkanes, ketones, and aromatics compounds. The change in the metabolite constituents of BGN malts showed that the steeping and sprouting time affected the

metabolites of BGN seeds, and the hypothesis that the malting process will affect the BGN metabolite was established.

Research chapter 5 achieved the third objective by establishing the physicochemical and biochemical characteristics of BGN speciality malt and syrups obtained from the optimum BGN malt. The Bambara groundnut speciality malts consisted of Base malt (BM) produced by drying sprouted BGN (green malt) at 50^{o}C for 24 h, toasted malt (TM) was produced from the base malt (dried at 50^{o}C) then subjected to 170^{o}C for 30 min. The colours of the speciality malts and syrups showed desirable colour characteristics in food industries. The evaluation of the colour coordinates in the study of speciality malt showed differences in colour at different temperatures with a decrease in lightness (L*) at high temperatures and increased redness (a*) and yellowness (b*) at higher temperatures. The pH of the Bambara groundnut speciality malts showed similarity to the pH of barley and could thus be a potential ingredient in the production of non-alcoholic beverages. The protein content of the Bambara groundnut speciality malts differs, while the protein content of the syrups was not. The amylase activities of the BGN speciality malts decreased due to the enzymes' thermosensitivity. The activities of the total polyphenols and antioxidants differed for all BGN speciality malts. Fifteen amino acids consisted of seven essential amino acids, and eight non-essential amino acids were detected. Fatty acid methyl esters identified were palmitoleic, oleic, linolelaidic, linoleic arachidic acid. There were 15 amino acids detected consisting of essential and non-essential amino acids. The sugars, organic acids and sugar alcohols consisted of lactic acid, fructose, sucrose, and myo-inositol. Twenty-nine volatiles were detected in the speciality malts with the pyrazine, 2,5-dimethyl, more abundant. The hypothesis that Bambara groundnut malt syrups produced from optimum amylase will exhibit good physicochemical characteristics was established and possessed beneficial components that can easily be incorporated into food formulation for human consumption for their health benefits.

The effect of the malting process on BGN seeds thereby brought about the speculation that the BGN malts and their syrups will encourage the production of BGN in large quantities by the farmers, improve the livelihood of the predominantly female farmers, and improve the economic condition of the country by facilitating international trade of the indigenous crop. Hence, the new BGN speciality malts and their syrups could be used as functional ingredients and condiments in households and industrial levels, eliminating the laborious process of BGN in food products and beneficial to health-conscious consumers.

The following conclusions can therefore be drawn from this study:

1. Steeping and sprouting times had effects on the physicochemical characteristics of BGN seeds.
2. The total polyphenolic and antioxidant contents of Bambara groundnut seeds improved with steeping and sprouting times.
3. Steeping at 36 h and sprouting for 96 h gave the optimum α- and β- amylase rich Bambara groundnut malt.
4. The malting process (steeping and sprouting times) affected the Bambara groundnut metabolites.
5. The metabolite profiling using GC-MS (Gas Chromatography-Mass Spectrometry), GC-FID (Gas Chromatography-Flame Ionization Detection), HS-GC-FID (Headspace Gas Chromatography-Flame Ionization Detector) to track the metabolites changes during the BGN malting process was suitable for detecting the lipophilic and hydrophilic low molecular weight constituents consisting of the FAME, amino acids, volatiles, and sugars, sugar alcohol, and acids.
6. The metabolites detected from the malted BGN seeds exhibited characteristics properties that can be applied in food industries.
7. The speciality BGN malts and syrups produced from optimum amylase activities exhibited desirable physicochemical and biochemical characteristics.
8. The physicochemical characteristics including, the colour, pH, ^{o}Brix, protein content, amylase, total polyphenolic and antioxidant activities of speciality BGN malts and syrups, make them suitable for condiments in homes, food, and beverage industries.

The metabolite profile of the Bambara groundnut malting process showed that the metabolic changes during the malting process were time-dependent. Therefore, further investigations into the quality of the metabolites in this study are still needed. This would provide a better understanding of the Bambara groundnut malt and assist in providing an insight into these metabolites that could be beneficial to health with desirable functional characteristics. In addition, the malted Bambara seeds and the syrups still need to be used in the production of food and beverages to assess their functionality and consumer acceptability.

Appendices

Appendix A: Approved Ethical Clearance

1. Ethics exception letter

Cape Peninsula University of Technology

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

Ethics exemption Letter **Reference no: 218078951/01/2020**

Office of the Chairperson **Research Ethics Committee**	**Faculty of Applied Sciences**

On 31 January 2020, the Faculty Research Ethics Committee of the Faculty of Applied Sciences determined that the research activities related to a project to be undertaken by Adetokunboh, AH for a degree (MASTER OF FOOD SCIENCE AND TECHNOLOGY) at the Cape Peninsula University of Technology does not require ethics clearance. The ethics exemption for the project is approved.

Title of project:	Metabolite profile of Bambara groundnut malting process and characteristics of its malt extracts

Comments (Add any further comments deemed necessary, e.g. permission required)

1. Human subjects are not included in the proposed study.
2. This permission is granted for the duration of the study.
3. Research activities are restricted to those detailed in the research proposal.
4. The research team must comply with conditions outlined in AppSci/ASFREC/2015/1.1 v1, CODE OF ETHICS, ETHICAL VALUES AND GUIDELINES FOR RESEARCHERS.

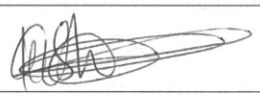
Signed: Chairperson: Research Ethics Committee	31/01/2020 **Date**

2. Statement of permission

Cape Peninsula University of Technology

Statement of Permission

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

Reference no.	218078951/01/2020
Surname & name	Adetokunboh, AH
Student Number	218078951
Degree	MASTER OF FOOD SCIENCE AND TECHNOLOGY
Title	Metabolite profile of Bambara groundnut malting process and characteristics of its malt extracts
Supervisor(s)	PROF VICTORIA ADAORA JIDEANI
FRC Signature	
Date	2020/02/04

Appendix B: Book of Abstracts – Research Output Presented at National and International Conferences

1. Poster presentation: 6th International ISEKI-FOOD Conference: Effect of steeping and sprouting times on amylase activities of *Vigna subterranea* seeds.
2. Poster presentation: CPUT Postgraduate Conference 2021: Effect of steeping and sprouting times on total phenolic content and antioxidant activities of *Vigna subterranea*.
3. Article awaiting publication in accredited journal Heliyon: Effect of steeping and sprouting times on the metabolites of malted Bambara groundnut

3. Article published in accredited MDPI journal: Enzyme and Antioxidant activities of Malted Bambara Groundnut as Affected by Steeping and Sprouting Times

foods

MDPI

Article

Enzyme and Antioxidant Activities of Malted Bambara Groundnut as Affected by Steeping and Sprouting Times

Adeola Helen Adetokunboh, Anthony O. Obilana and Victoria A. Jideani *

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Abstract: Bambara groundnut (BGN) is termed a complete food due to its nutritional composition and has been researched often for its nutritional constituents. Malting BGN seeds have shown improved nutritional and functional characteristics, which can be used to produce an amylase-rich product as a functional ingredient for food and beverage production in homes and industries. The aim of this study was to investigate the enzyme and antioxidant activities of malted BGN affected by steeping and sprouting times. BGN was malted by steeping in distilled water at 25–30 °C for 36 and 48 h and then sprouted for 144 h at 30 °C. Samples were drawn every 24 h for drying to study the effect of steeping and sprouting times on the moisture, sprout length, pH, colour, protein content, amylase, total polyphenols, and antioxidant activities of the BGN seeds. The steeping and sprouting times significantly affected the BGN malt colour quality and pH. The protein content of the malted BGN seeds was not significantly different based on steeping and sprouting times. Steeping and

Appendix C: Poster Second Prize at the 2021 Postgraduate Conference

Congratulations: Prize winner at CPUT Postgraduate Conference

Cape Peninsula University of Technology

Centre for Postgraduate Studies
Bellville Campus P O Box 1906
Bellville
7537
Tel: 021 953 8455

Email: burgerd@cput.ac.za

7 December 2021
Dear Adeola Helen Adetokunboh

Re: Offer of CPUT Postgraduate Conference Prize: Adeola Helen Adetokunboh (218078951)

Congratulations, once again, on winning the Poster Second Prize at the 2021 Postgraduate Conference.

This letter serves to inform you that, as a Second Prize winner for Poster Presentations at the 2021 Annual CPUT Virtual Postgraduate Conference you have been awarded a voucher from Van Schalk's to the value of R5 000.00.

Please email us where you are currently staying, so that we can arrange for you to receive (or collect) your prize.

Yours Sincerely

Director: CPGS
Prof Dina Burger,
On behalf of the Conference Committee