

THE EFFECT OF DIFFERENT PROCESSING METHODS ON THE NUTRITIONAL,  
ANTI-NUTRITIONAL AND EMULSIFYING PROPERTIES OF INOCULATED AND NO-  
INOCULATED BAMBARA GROUNDNUT (*Vigna subterranea*) CULTIVARS

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE  
MASTER OF SCIENCE IN AGRICULTURE (FOOD SCIENCE AND  
TECHNOLOGY)

OF

THE UNIVERSITY OF NAMIBIA

BY

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OCTOBER 2024

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## **Abstract**

Bambara groundnut (*Vigna subterranea*) is one of the Namibian underutilised legumes that provides protein, carbohydrates, fats, potassium, magnesium, phosphorus, zinc and iron. Several researchers suggest that *Bradyrhizobium* inoculation has the capacity to improve nutritional composition of Bambara groundnut. This study evaluated the effects of processing on the nutritional composition, anti-nutritional factor (ANF) and emulsifying property of inoculated and non-inoculated cream, brown and red Bambara groundnut cultivars that were grown in Namibia. The cultivars were roasted for 15, 30 and 45 minutes at a temperature of 140°C respectively and boiled for 30 minutes in a solution of 0.5g/100mL of sodium bicarbonate, sodium chloride, and magnesium sulphate respectively. The beans were blended to flours and analysed. Results indicate that each boiling salt has a unique effect on each nutritional component. Certain nutritional composition increased with an increase in roasting time. Bambara groundnut inoculated with *Bradyrhizobium* had higher protein and emulsifying properties. Fat content of all inoculated Bambara groundnut was lower after processing. Red Bambara groundnut had the highest fat and protein while brown and cream Bambara groundnut had a higher crude fibre and carbohydrate. Non-inoculated Bambara groundnut had lower minerals compared to other Bambara groundnut reported in other studies. Tannins could not be detected in the Bambara groundnut experimented in this study.

**Keywords:** Anti-nutritional Factors (ANF), Bambara groundnut (BGN), Boiling, Inoculation, Roasting

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## **List of Publications from Conference Proceedings**

### **Conference Papers**

Kapembe, M.N.P, Hiwilepo van Hal., P. and Jideani, V. *The effect of different processing methods on the nutritional composition, anti-nutritional factors and emulsifying properties of inoculated and non-inoculated Vigna subterranea (Bambara groundnut)*. PowerPoint presentation at the Annual Research Conference on the Agriculture, Engineering and Natural Sciences (ARCAENS), 23-24 November 2022, University of Namibia.

### **Research Seminars**

Kapembe, M.N.P, Hiwilepo van Hal., P. and Jideani, V. *The effect of different processing methods on the nutritional composition, emulsifying property and anti-nutritional factors of inoculated Vigna subterranea (Bambara groundnut)*. PowerPoint presentation at the Sustainable solutions to elevate local protein-rich crops from subsistence to high-tec innovative products Research Seminar, 25 November 2021, University of Bremen.

Kapembe, M.N.P, Hiwilepo van Hal., P. and Jideani, V. *Opportunities of Vigna subterranea (Bambara groundnut) to promote food security in Namibia*. PowerPoint presentation at the Sustainable solutions to elevate local protein-rich crops from subsistence to high-tech innovative products Research Seminar, 04-08 December 2023, TTZ Bremerhaven.

**Papers submitted for publishing**

Effect of *Bradyrhizobium* inoculation on the proximate, mineral, emulsifying activity and condensed tannins of Namibian grown Bambara groundnut flour. Welwitschia International Journal of Agricultural Sciences.

**Overview of completed trainings**

Microbiology in agricultural food production. Namibia University of Science and Technology, November 2021.

Sustainable solutions to elevate local protein-rich crops from subsistence to high-tech innovative products Research Seminar, December 2023.

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## **List of Abbreviations and Acronyms**

<b>AMTA</b>	Agro Marketing Trade Agency
<b>ANFs</b>	Anti-Nutritional Factors
<b>ANOVA</b>	Analysis of Variance
<b>BGN</b>	Bambara groundnut
<b>CT</b>	Condensed Tannins
<b>DAAD</b>	Deutscher Akademischer Austauschdienst
<b>EA</b>	Emulsifying Activity
<b>HTC</b>	Hard to cook
<b>IN</b>	Inoculated
<b>IRAD</b>	Institute of Agricultural Research for Development
<b>MANOVA</b>	Multivariate Analysis of Variance
<b>MgSO<sub>4</sub></b>	Magnesium Sulphate
<b>NaCl</b>	Sodium Chloride

<b>NaHCO<sub>3</sub></b>	Sodium Bicarbonate
<b>NIOS</b>	Namibia Institute of Seeds
<b>NO</b>	Non-inoculated
<b>RD</b>	Resistant Starch
<b>USA</b>	United States of America

## **Acknowledgment**

First and foremost, I would like to give all the glory to the LORD Jesus Christ who in His mercy saw me fit to be a candidate for this study.

All appreciation goes to Dr Penny Hiwilepo van Hal, my supervisor who poured out her efforts to ensure I complete this study. I would also like to wholeheartedly appreciate my Co-Supervisor Professor Jideani Victoria who assisted me in every manner possible to ensure I excel in this study. Their corrections and timely advice aided me to get this point.

I would not have accomplished this study without the spiritual, emotional, mental, financial and academic support of my beloved parents, leaders and Y4C family.

I would like to appreciate Ms Kanime Welhemina and Ms Nekulu who tirelessly offered endless assistance.

I thank DAAD and the coordinators who funded this study.

## **Dedication**

This thesis is dedicated to my Father, El Shaddai and the institution of LCCC Youth For Christ who without their initiating investment into my life, I would not have been able to complete this study.

## Declarations

I, Maria N.P Kapembe, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof, has not been submitted for a degree in any other institution of higher education. No part of this thesis may be reproduced, stored in any retrieval system, or transmitted in any form, or by means (e.g. electronic, mechanical, photocopying, recording or otherwise) without the prior permission of the author, or The University of Namibia on that behalf. I, Maria N.P Kapembe, grant The University of Namibia the right to reproduce this thesis in whole or in part, in any manner or format, which The University of Namibia may deem fit, for any person or institution requiring it for study and research; providing that The University of Namibia shall waive this right if the whole thesis has been or is being published in a manner satisfactory to the University.



Date: October 2024

Maria N.P Kapembe

## **Chapter 1: Introduction**

This chapter is composed of the introduction and background of the study. Study questions, hypotheses and significance of the study are also stated in this chapter.

### **1.1 Background of the study**

According to Bjornlund (2020), it has been suggested that agricultural activities possess the potential to not only increase agricultural production and income, but they also possess the capability to significantly contribute to the improvement of food security. It is postulated that food security may be effectively enhanced through the implementation of strategies aimed at increasing the production and processing of underutilised crops. *Vigna subterranea*, commonly known as Bambara groundnut, represents one such underutilised leguminous crop that is cultivated across the northern regions of Namibia (Mukakalisa, 2010). It is worth noting that the groundnut exhibits a wide array of colours, including brown, dark brown with black dots, yellow with brown patches, red, and cream (Adeleke, Adiamo, & Fawale, 2018). It is of paramount importance to acknowledge that Namibia is home to these variants of Bambara groundnuts (Mukakalisa, 2010).

Currently, in Namibia, there is a scarcity of comprehensive information pertaining to the botanical characteristics of the Bambara groundnut cultivars. Currently, the classification of this legume is presently based solely on the colour of its coat. While a number of studies have indicated that *Vigna subterranea* is abundant in both protein and carbohydrates, and thus may possess the potential to be utilised within the food industry due to its functional properties (Mamphosa & Jideani, 2016; Nwadi, Uchebu & Okonkwo, 2019), it is worth noting that there exists a limited amount of knowledge with regard to how various processing methods may

impact the nutritional composition, emulsifying capability, and Anti-Nutritional Factors (ANFs) within the context of Namibia. Moreover, it is important to acknowledge that the realm of academia has witnessed a burgeoning interest in the exploration of inoculating crops with bacteria to enhance crop yield and foster the development of drought-resistant crops (Silva et al., 2017). Despite the considerable potential advantages associated with these endeavours, there remains a vast reservoir of untapped knowledge concerning the advantageous utilisation of processed microbially inoculated legumes within the confines of Namibia.

### **1.2 Statement of the problem**

Even though numerous studies have been conducted on processed Bambara groundnut in other parts of Africa, information on the effects of various processing methods on the nutritional and emulsifying properties of this legume in Namibia is limited (Fleißner, 2009). As a result, it is imperative to carry out research in Namibia to address this knowledge gap and provide valuable insights on the impact of different processing techniques on the nutritional and emulsifying properties of Bambara groundnut. This will contribute significantly to the existing body of literature on this topic and help researchers, policymakers, and nutritionists make informed decisions regarding the consumption of Bambara groundnut in Namibia.

Moreover, a study conducted in Nigeria by Ijarotimi & Esho (2009) revealed that processing led to a reduction in Anti-Nutritional Factors (ANFs) in Bambara groundnut. However, the current scenario in Namibia depicts a discouragement of Bambara groundnut consumption among children due to the perceived presence of ANFs. The presence of ANFs is believed to create a bitter taste in the legume which discourages consumers from eating it (Lin Tan et al., 2020; Ramatsetse, Ramashia, & Mashau, 2023). However, in Namibia there is limited research

to explain this phenomenon. This highlights the urgent need to assess the efficacy of various processing methods in reducing ANFs in Bambara groundnut. By doing so, we can determine the extent to which processing can alleviate concerns surrounding ANFs and potentially enhance the acceptance and consumption of this nutritious legume among Namibian children. Therefore, conducting a comprehensive investigation on the effects of processing methods on the nutritional and emulsifying properties of Bambara groundnut in Namibia is of utmost importance. This research will shed light on the potential benefits of processing in reducing ANFs, which will not only address the current gap surrounding Bambara groundnut consumption but also promote its utilisation as a valuable dietary resource for Namibian children.

Moreover, in Namibia, there is a lack of available information pertaining to the nutritional advantages associated with the consumption of legumes that have been inoculated with *Bradyrhizobium* bacteria. The practice of inoculation is currently recognized as a discipline that assists farmers in achieving enhanced crop yields and cultivating crops that are resistant to pests. As a result, the findings derived from this study will provide valuable guidance to both tertiary institutions and the government, informing them of the necessity to allocate additional resources towards conducting further investigations, providing comprehensive training programs, and organising workshops, all with the ultimate objective of successfully implementing the method of inoculation for Bambara groundnut. By doing so, it is anticipated that this approach will significantly contribute towards the improvement of food security in Namibia. Additionally, the findings of this study will contribute to the existing scientific knowledge on the subject and serve as a valuable resource for future research endeavours in this field.

### **1.3 Objectives**

The aim of this study was to evaluate the effects of boiling and roasting on the nutritional content, emulsifying property and anti-nutritional factors of non-inoculated and inoculated Bambara groundnut and subsequently determining which cultivar can be used to produce alternative food products based on its beneficial functional properties.

The specific objectives of the study were:

1. Investigate the effect of boiling with alkaline salts on the nutritional content, emulsifying property and anti-nutritional factor of three different non-inoculated and inoculated Bambara groundnut cultivars.
2. Assess the effect of roasting at different times on the nutritional content, emulsifying property and anti-nutritional factor of three different non-inoculated and inoculated Bambara groundnut cultivars.

### **1.4 Hypotheses**

1. There is no difference in the nutritional content, emulsifying property and anti-nutritional factor of three different non-inoculated and inoculated Bambara groundnut (*Vigna subterranea*) cultivars boiled using alkaline salts.
2. There is no difference in the nutritional content, emulsifying property and anti-nutritional factor of three different non-inoculated and inoculated Bambara groundnut (*Vigna subterranea*) cultivars roasted at different times.

### **1.5 Significance of the study**

This study had the primary objective of imparting knowledge and enlightening the Namibian population about the advantageous nutritional aspects that are closely associated with the consumption of processed Bambara groundnut. Additionally, the findings and outcomes derived from this study aim to furnish valuable and informative data to the various stakeholders within the food industry regarding the emulsifying properties exhibited by each distinct groundnut cultivar when subjected to the process of processing. Moreover, the results that have been obtained from this comprehensive study effectively outline and delineate the essential knowledge and information pertaining to the profound impact and effects of employing microbial techniques in cultivating and handling Bambara groundnut including other Namibian grown legumes, thereby providing invaluable insights to researchers and agriculturalists alike.

### **1.6 Limitations of the study**

One of the primary restrictions encountered during this study was the constraint of having a limited amount of time available, as well as a restricted allocation of research funds, which ultimately hindered the ability to explore alternative processing methods and alternative ANFs. Moreover, the limitation of having a finite number of samples of inoculated Bambara groundnut further contributed to the constrictions faced in terms of the extent of analysis that could be undertaken. It is worth mentioning that due to the absence of any additional funding, the opportunity to conduct surveys with the aim of gathering insights into the traditional and indigenous utilisation of Bambara groundnut was not completed.

### **1.7 Delimitation of the study**

The analysis conducted in this study exclusively focused on non-inoculated Bambara groundnut that were acquired from the Namibia Institute of Seeds (NIOS). No samples

gathered from other regions of Namibia were utilised in this investigation. Furthermore, it should be emphasised that inoculated Bambara groundnut was provided by a postgraduate student affiliated with the University of Namibia.

## **Chapter 2: Literature Review**

This chapter looks at the review of origin, taxonomy and plant structure of Bambara groundnut. The various uses of the legume which range from human consumption, medicinal benefits, animal uses, and alternative uses are also discussed. The nutritional composition, bacterial inoculation and processing methods of Bambara groundnut are also discussed here.

### **2.1 Overview of Bambara groundnut (*Vigna subterranean*)**

#### **2.1.1 Origin**

*Vigna subterranea* (commonly known as Bambara groundnut) is a legume of African origin that has not received much attention in terms of its utilisation and value addition. This legume is cultivated in various regions of Africa, North America, and Asia, as documented by Ogundele (2014). It is believed that the crop originated from West Africa, with a particular emphasis on north-eastern Nigeria and north Cameroon. From these regions, it gradually spread across sub-Saharan and southern Africa, as stated by Maphosa, Jideani, and Maphosa (2022). Interestingly, the Bambara groundnut is commonly referred to by its name due to its association with the Bambara tribe, whose members predominantly reside in Mali (Heller, Begemann, & Mushonga 1997). This nomenclature reflects the historical and cultural ties between the legume and the tribe. Furthermore, the exact centre of origin of the Bambara groundnut is speculated to be situated in the geographical region that lies between West and Central Africa, which is near Timbuktu, a city located in Central Mali that holds historical significance (Majola, Gerrano, & Shimelis, 2021). The rich history and distribution patterns of the Bambara groundnut make it an intriguing subject for further study and exploration.

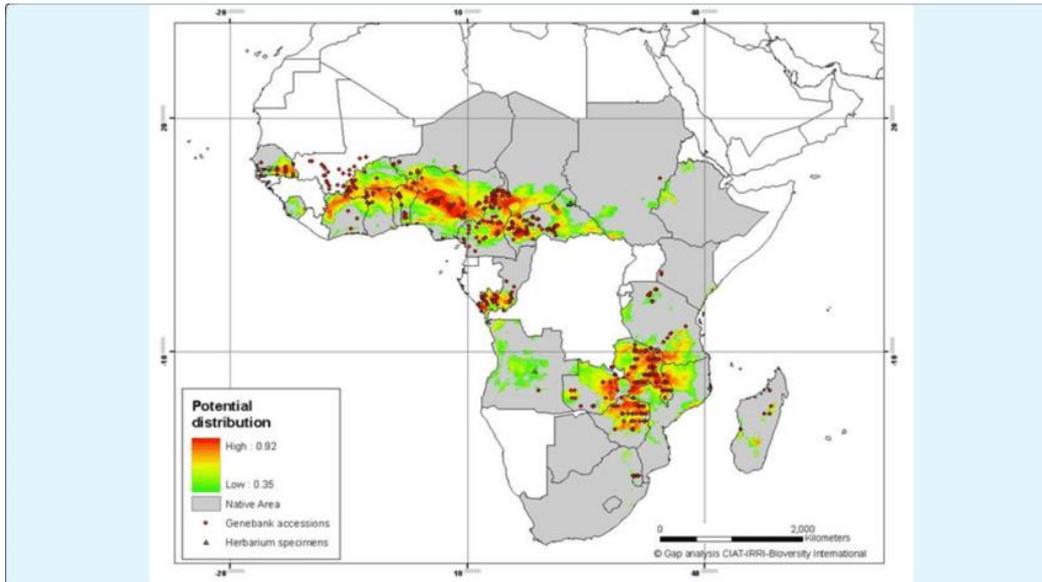


Figure 1 Distribution of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) in Africa (Temegne et al., 2018)

### 2.1.2 Taxonomy

In the 17th century, Linnaeus initially categorised Bambara groundnut in his work titled *Species Plantarum*, and named upon the *Glycine subterranea*, in accordance with his well-established system of nomenclature (Mkandawire, 2006; Temegne et al., 2018). However, during the subsequent 18th century, Du Petit-Thouars fortuitously discovered this crop in the region of Madagascar, subsequently denoting it as 'voandzou' in the French language where he consequently, proposed the alternative designation of *Voandzeia subterranea* (L.) (Heller, Begemann, & Mushonga, 1997). Following in this historical continuum, during the 19th century, a comprehensive investigation conducted by Maréchal, Mascherpa, & Stainier (1978) discovered significant similarities between peas and the genus *Vigna*. As a result of these findings, Verdcout verified the study and put forth the present-day nomenclature of *Vigna subterranea* (L.) Verdc (Temegne et al., 2018). It is noteworthy to mention that Bambara groundnut, belonging to the esteemed family of Fabaceae, holds the esteemed rank of being

the third most vital legume, following in the footsteps of the esteemed groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) (Ramatsitse, Ramashia, & Mashau, 2023). Thus, it is evident that the scientific classification of the Bambara groundnut, as eloquently classified by Temegne et al. (2018), is as follows:

- Kingdom: Plantae
- Sub Kingdom: Tracheobionta
- Class: Magnoliopsida (Dicotyledon)
- Sub Class: Rosidae
- Order: Fabales
- Family: Fabaceae
- Sub Family: Fabaceae Papilionoideae
- Tribe: Phaseoleae
- Subtribe: Phaseolinae
- Genus: *Vigna*
- Species: *Vigna subterranea* (L.) Verdc.

### **2.1.3 The plant**

Bambara groundnut, a plant characterised as herbaceous exhibits a unique growth pattern with its creeping stems that are situated at ground level (Gerrano, Eifediyi, Labuschagne, Ogedegbe, & Hassen, 2021). When observing the plant from an external perspective, one can discern a cluster of leaves that are tightly grouped together, forming an overall appearance of bunched foliage (Stephens, 1994). These leaves consist of internodes that emerge from branched stems, ultimately culminating in a crown-like formation on the surface of the soil (Heller, Begemann, & Mushonga, 1997). Additionally, it is noteworthy that Bambara groundnut possesses a well-

developed tap root system, which serves as an integral component of the plant's structure (Mateva, Chai, Mayes, & Massawe, 2022). This tap root system further gives rise to nodulating nodules that possess the remarkable ability to fix atmospheric nitrogen, contributing to the plant's overall nitrogen acquisition process (Lin Tan et al., 2020). This process is achieved by means of a mutually beneficial relationship established with a specific bacterium known as *Rhizobium* bacteria which operates by replacing the nitrogen present in the soil, thereby resulting in a substantial increase in crop yields (Fwanyanga, Horn, Sibanda & Reinhold-Hurek, 2022). This phenomenon, as clarified by Gqaleni (2014) and Heller, Begemann, & Mushonga (1997), reveals the intricate nature of this symbiotic bond between the plant and the bacterium. In the final stages of the plant's life cycle, it produces flowers that typically exhibit a light brown hue which are characterised by the presence of a calyx, which is composed of five sepals covered in fine hairs (Chandra, Nandini, Pranesh, & Gobu, 2017). Gqaleni (2014) and Heller, Begemann, & Mushonga (1997) provide further insights into the appearance and composition of these flowers. The two above mentioned authors reported that the calyx itself undergoes development as the pod of the plant grows and matures underground. The pods may contain either a single seed or two seeds, with certain varieties found in Congo even exhibiting the presence of three seeds (Heller, Begemann, & Mushonga 1997). Majola, Gerrano, and

Shimelis (2021) provide evidence of this interesting variation in seed quantity within the pods.

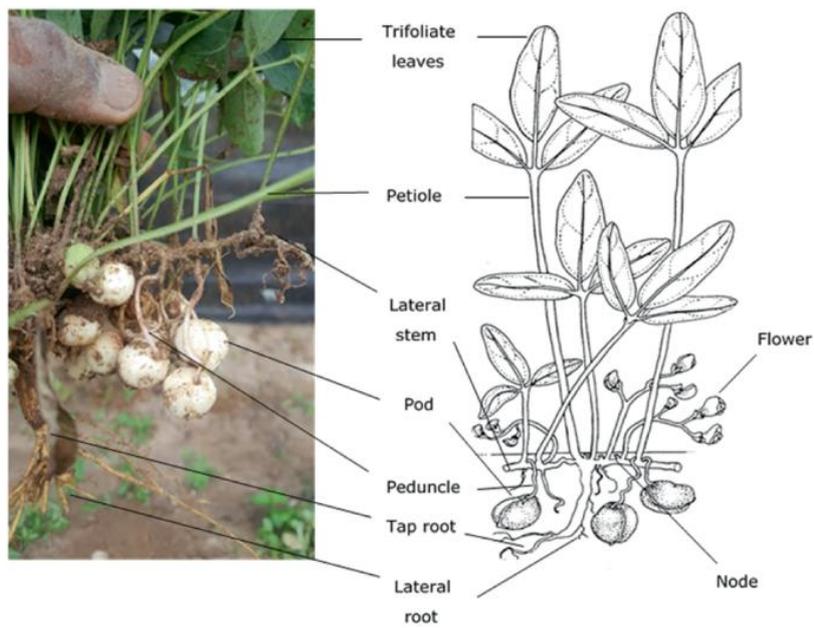


Figure 2 Different parts of a Bambara groundnut plant (Mayes et al., 2019)



Figure 3 Bambara groundnut plant (Tlankka, Mbega, & Ndakidemi, 2020)



Figure 4 Harvested Bambara groundnut pods (Mubaiwa, 2018)



Figure 5 Bambara groundnut seeds (Jideani, 2016)

Bambara groundnut exhibits the remarkable ability to endure periods of drought and thrive in soils of poor quality; however, it has been documented in a study conducted by Temegne et al (2018) that this resilient legume can also withstand heavy rainfall events. When examining the physical characteristics of Bambara groundnut seeds, it is apparent that they possess irregularities in both size and shape, with an average length falling within the range of 11.01mm to 11.81mm (Mpotokwane, Gaditlathelwe, Sebaka, & Jideani, 2008). In an intriguing investigation conducted by Heller, Begemann, & Mushonga (1997), it was observed that the coat colour of Bambara groundnut seeds can vary significantly, encompassing a spectrum spanning from white to creamy, yellow, brown, purple, red, or black. The above-mentioned author also reported that the testa patterns exhibited by these seeds can manifest as mottled, blotched, or striped, or they can be uniformly coloured. These findings are depicted in figure 4.

Bambara groundnut has been greatly overlooked and not given much attention. As a result, there is a severe lack of data available when it comes to the production, processing and harvest volumes of this legume in Namibia. The information that is available is extremely limited. According to reports from 2014, the annual world production of this legume was reported to be 330,000 tonnes. It is worth noting that a significant portion, approximately 40-50%, of this production is concentrated in West Africa, specifically Nigeria (Alhassan & Egbe, 2014). In Namibia, the production of this legume is mainly concentrated in certain regions, namely Oshana, Oshikoto, Omusati, Ohangwena, Kavango East, and Kavango West. However, the yields in these regions are quite low, ranging from only 300 to 800 kg per hectare (Mukakalisa, 2010). These low yields are concerning because they do not meet the market requirements. As a result, there is a need for additional production to meet the demand. Interestingly, a

considerable amount of Bambara Groundnut is informally imported from Southern Angola and then sold alongside the local harvest on traditional and informal markets (Fleißner, 2006). This practice highlights the challenges faced in meeting the demand for this legume in Namibia and the need for further research and development to improve production and harvest volumes.

Table 1 Production of Bambara groundnut of top leading countries in Africa (FAOSTAT, 2023)

<b>Country</b>	<b>Estimate production (tonnes)</b>
Burkina Faso	45 930
Niger	25 854
Cameroon	23 422
Mali	20 201
Zimbabwe	19 812
Togo	10 149
Dr Congo	9 628
Zambia	4 674

#### **2.1.4 Uses of Bambara groundnut**

To this day, there are numerous reports available that provide a comprehensive understanding of the various ways in which Bambara groundnut is utilised throughout the vast continent of Africa. The primary utilisation of Bambara groundnut predominantly revolves around its consumption by humans, specifically as a food source. However, it is worth noting that there have been documented reports that shed light on the diverse applications of this versatile crop,

including its medical usage, its incorporation as a component in animal feed, and its potential for being transformed into innovative and novel food products.

#### **2.1.4.1 Human consumption**

The seeds can be consumed in two stages of their development, either when they are immature or when they are fully ripe, as discussed by Majola et al (2021). When the seeds are in their immature stage, they offer multiple options for consumption. They can be savoured fresh, boiled with the addition of salt and pepper, or even grilled, as highlighted by Akande et al (2009) and Heller et al (1997). Additionally, the pods of the seeds, when still green and in their early stages of growth, can be harvested and utilised as a vibrant green vegetable, as discussed by Tegmene et al. (2018). On the other hand, when the seeds reach their mature stage, they become ripe, dry, and hard, requiring some preparation before they can be consumed. In this case, the dry seeds can be processed in two different ways. Firstly, they can be pounded to obtain flour, which is then boiled to create a thick and hearty porridge or soaked in water and subsequently boiled, as indicated by Mubaiwa et al (2018) and Heller et al (1997). Furthermore, dried seeds can also be combined with maize or plantain and boiled, resulting in a flavourful and nutritious dish, as suggested by Alhassan et al (2014) and Tegmene et al (2018).

To this point in time, a wide range of processed Bambara groundnut products have been effectively manufactured. In the country of Zambia, there have been reports of the creation of bread using Bambara groundnut flour, whereas in Ghana, the commercial canning of Bambara groundnut in gravy has proven to be a thriving industry (Mukakalisa, 2010; Akande et al., 2009). In Benin, the seeds are skilfully ground into flour to produce appetising cakes and dumplings (Tegmene et al., 2018). In the regions of Eastern Africa, Bambara groundnuts are

utilised as a viable substitute for coffee (Mubaiwa et al., 2018). Despite the relatively low oil content, certain tribes in Congo cleverly roast the seeds of Bambara groundnut and extract its precious oil (Heller et al., 1997). Bambara groundnut possesses the potential to serve as a valuable ingredient in the fortification process of cereal-based food products. This fortification strategy aims to enhance the overall nutrient composition of both baby and adult pap and pottage, thereby contributing to improved nutritional value (Akande et al., 2009). In a recent research trial conducted by Heller et al (1997), it was revealed that Bambara groundnut milk emerged as the preferred choice due to its aesthetically pleasing lighter colour when compared to alternative milk sources such as cowpea milk, pigeon milk, and soybean milk. This finding highlights the significance of Bambara groundnut milk as a viable option for utilisation in various African countries as a weaning food (Majola et al., 2021). For instance, in Cameroon, the Institute of Agricultural Research for Development (IRAD) incorporates Bambara groundnut flour into cereals during the manufacturing process of a popular porridge known as "baby lion" porridge (Tegmene et al., 2018). Similarly, in South Africa and Swaziland, the utilisation of Bambara groundnut seeds to enhance the flavour profile of boiled cowpea grain has been observed (Majola et al., 2021). These diverse applications and culinary practices highlight the versatility and potential of Bambara groundnut as a valuable resource in the context of food fortification. In the Namibian context, the consumption of Bambara groundnut has not yet been extensively documented. However, a survey in Kavango East region by Fwanyanga (2023) documented that Bambara groundnut can be used to prepare the following various traditional dishes:

- Once Bambara groundnut is boiled for about an hour, they are directly consumed as a snack.

- Dry Bambara groundnut seeds can be boiled and mixed with *mahangu* flour to make a stiff porridge called "mugombakapindi".
- Bambara groundnut can also be directly prepared to make a relish or soup.
- Dry Bambara groundnut can be roasted and complemented with peanut flour to form a snack called "shikuvira".
- Bambara groundnut may be boiled in a dry or fresh state and mixed with corn
- Dry Bambara groundnut may be roasted, crushed into flour and mixed with warm water to prepare instant porridge called "rutura".

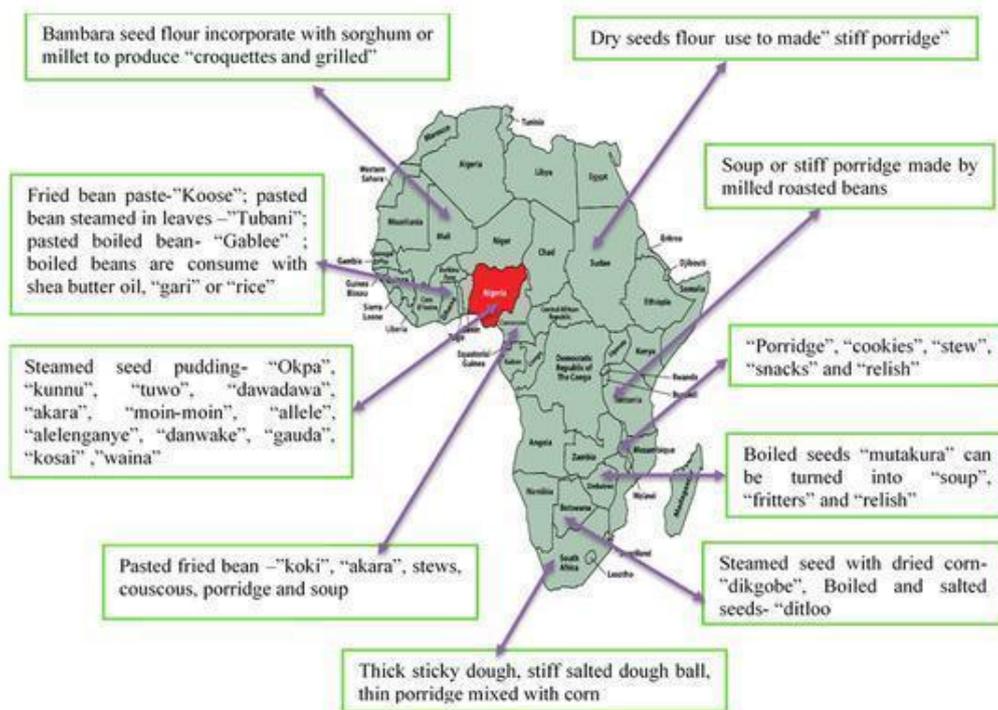


Figure 6 Value added products of Bambara groundnut across Africa (Khan, Raffi, Ramler, Jusoh, & Al-Mamun, 2021)

#### **2.1.4.2 Medicinal use**

Traditionally, the utilisation of Bambara groundnut has been employed in the management and alleviation of diverse physical ailments. This legume possesses inherent medicinal properties, as highlighted by Valombola et al. (2019). In Senegal, the concoctions derived from the leaves of Bambara groundnut are utilised for the treatment of abscesses and infected wounds (Udeh, Nyila, & Kanu, 2020). Furthermore, the research by Udeh, Nyila, & Kanu (2020) states that the extracted juice from these leaves is applied topically to the eyes to address the symptoms associated with epilepsy. Moreover, the roots of this legume are occasionally used as an aphrodisiac, while the crushed seeds, when mixed with water, are administered for the treatment of cataracts (Tegmene et al., 2018). Additionally, Majola et al (2021) highlights that the boiling of white Bambara groundnuts in conjunction with guinea fowl meat has been observed as an effective measure to combat diarrhoea. Similarly, the utilisation of black Bambara groundnuts, when mixed with water, has shown promising potential in the treatment of sick children (Okafor, Jideani, Meyer, & Le Roes-Hill, 2022). The tribe of Igbo in Nigeria utilises the plant for the purpose of treating venereal diseases while in the neighbouring country of Cameroon, the indigenous people rely on Bambara groundnut as a remedy against amoebic dysentery, sore throat, headaches, stomach pain, joint pain, and even bone decalcification (Tegmene et al., 2018). The consumption of Bambara groundnut aids in the process of digestion due to its possession of laxative properties as it has been observed that Bambara groundnut possesses the ability to stimulate milk production in lactating women, serving as a valuable dietary supplement (Ramatssetse, Ramashia, & Mashau, 2023). Additionally, women who have recently given birth are encouraged to consume this Bambara groundnut as it facilitates the healing of their wounds (Tegmene et al., 2018). Furthermore, the use of Bambara groundnut

flour has proven effective in the treatment of skin rashes, while the seeds themselves are often chewed to alleviate the discomfort caused by a swelling jaw (Majola et al., 2021). It is worth noting that Tegmene et al (2018) have also highlighted the presence of kaempferol, an antioxidant polyphenol, within the seeds of Bambara groundnut. This compound is known to significantly reduce the risk of numerous chronic diseases, including cancer.

#### **2.1.4.3 Animal feed**

The utilisation of Bambara groundnut and its by-products as livestock feed offers a variety of advantages. It has been noted that the haulm, which refers to the stems and leaves of the plant, contains high levels of nitrogen and phosphorus which make these compounds make the leaves highly suitable for animal grazing, thereby contributing to the nutritional well-being of livestock (Alhassan & Egbe, 2014; Mojola et al., 2021; Heller et al., 1997; Ibny, Jaiswal, Mohammed, & Dakora, 2019). In a study conducted by Majola et al (2021), it was observed that in Nigeria, tilapia fish are provided with protein derived from the leaves of Bambara groundnut. This finding further emphasises the versatility of this crop in animal nutrition. Moreover, the seeds of Bambara groundnut can also serve as a valuable source of feed for other animals such as pigs and poultry (Tegmene et al., 2018; Volombola et al., 2019; Mukakalisa, 2010). The successful utilisation of processed Bambara groundnut as a feed for livestock animals, including rabbits, has been demonstrated by various researchers (Akande et al., 2009). Hence, it is evident that the potential of Bambara groundnut and its by-products as livestock feed is vast and holds great promise for the agricultural industry.

#### **2.1.4.4 Other uses**

This leguminous crop serves a valuable purpose in the practice of crop rotation, as it plays a crucial role in replenishing the soil with essential nitrogen. This process occurs through the fascinating mechanism of nitrogen fixation, which has been extensively studied and documented by Mukakalisa (2010). It is worth noting that in certain Namibian communities, the dried peels/shells of the pods of this crop are utilised as a source of firewood. Bambara groundnut shells are thrown back into the ground as fertilizer. During harvest, some seeds of Bambara groundnut are kept to be used for the next planting season. In Namibia through direct observation, it was found that fresh, boiled or dried Bambara groundnut is sold at Agro Marketing and Trade Agency (AMTA) or informal markets for N\$5 - N\$10/1L and N\$500 - N\$800/50kg. Bambara groundnut cannot be sold for a high profit because of the competition that exists amongst several informal traders. Despite the numerous potential benefits that this remarkable crop offers in terms of nutrition, medicine, and agronomy, it is disheartening to observe the lack of scientific attention and exploration that the Bambara groundnut has received thus far particularly in Namibia. This unfortunate neglect has been pointed out and discussed by Mubaiwa et al (2018) in their comprehensive research study.

## **2.2 Composition of Bambara groundnut (*Vigna subterranean*)**

### **2.2.1 Nutritional composition of Bambara groundnut (*Vigna subterranean*)**

In a recent investigation conducted by Adeleke et al (2018), it was revealed that legumes are currently being regarded as crucial components of the human breakfast, displacing cereals from their long-held position. One legume that has garnered attention as a potential substitute is the

Bambara groundnut, owing to its exceptional nutritional profile. This legume, which is characterised by its high nutrient density, has even earned the title of a "complete food" due to its meticulously balanced macronutrient composition, as reported by Lin Tan et al (2020). The above-mentioned authors further expound upon the composition of Bambara groundnut highlighting its remarkable contribution as a rich source of crude protein, carbohydrates, fats, and a commendable assortment of essential and non-essential amino acids, particularly lysine and methionine. In fact, Gqaleni (2014) and Ogundele (2016) reported that the dry basis of this legume boasts an impressive content range of 2.9-6.8% lysine and 1.3-1.8% methionine. Furthermore, an examination of the mineral content within Bambara groundnut reveals the presence of potassium, magnesium, phosphorus, zinc, and iron in significant quantities, surpassing the levels observed in other legumes such as chickpeas and mung beans (Lin Tan et al 2020). However, it is important to acknowledge that Bambara groundnut contains its share of anti-nutrients, including phytate, tannin, and oxalate, as meticulously documented by Nwadi et al (2019). These anti-nutritional factors (ANFs), have the potential to detrimentally impact the bioavailability of nutrients, as indicated by Lin Tan et al. (2020). Considering these findings, it is evident that the investigation of legumes, specifically Bambara groundnut, warrants further exploration to fully understand their implications in the realm of human nutrition.

Table 2 Nutrient comparison of various legumes (Hillocks, Bennet, & Mponda, 2012)

Various legumes						
Proximate (%)	Bambara groundnut	Soya	<i>Phaseolus</i> bean	Cowpea	Faba bean	Chickpea
<b>Protein</b>	21.8	36.5	23.8	23.6	26.1	19.3
<b>Carbohydrates</b>	61.9	30.2	59.6	60.0	58.3	60.6
<b>Fat</b>	6.6	19.9	2.1	0.8	5.7	6.0

### 2.2.1.1 Protein

Proteins, which are crucial constituents of the human diet, play a vital role in the advancement and expansion of human tissue. In terms of protein composition, Bambara groundnuts have been extensively documented to possess substantial quantities of proteins. The abundance of protein content in Bambara groundnuts implies that these legumes have the potential to emerge as a prominent source of protein in developing nations, where access to proteinaceous products is often hindered by economic constraints (Okafor, Jideani, Meyer, & Le Roes-Hill, 2022). Moreover, the protein content present in Bambara groundnuts signifies that they can serve as a feasible alternative protein source for individuals adhering to vegan or vegetarian diets. In addition to providing energy due to the presence of carbohydrates, the protein content in Bambara groundnuts constitutes a significant component of legumes. It is worth noting that the protein content in Bambara groundnuts may vary depending on the specific cultivar and geographical location (Traoré-Barro et al., 2023). Remarkably, the protein content in Bambara groundnuts is as noteworthy as that found in Marama beans and cowpeas (Baptista et al., 2017). Elucidating the diverse perspectives presented by different authors, the protein content in

Bambara groundnuts has been reported to range between 19-70% (w/w) (Yang et al, 2022; Busu, & Amonsou, 2019). The high content that is found in the given food product suggests that it possesses the ability to fortify or enhance the nutritional value of food items that have a relatively low protein content. This assertion is supported by a study conducted by Yang et al. (2022), wherein it was emphasised that Bambara groundnuts exhibit a significant richness in amino Acids, which are typically found to be insufficient in other types of plant proteins. Furthermore, it was noted that the amino acid profile of Bambara groundnuts bears a resemblance to that of soybean. Additional research conducted by Adebowale et al (2011) shed light on the fact that aspartic acid, glutamic acid, leucine, arginine, and lysine are among the most abundant amino acids found in Bambara groundnuts, while sulphur-containing acids, such as methionine, are present in the least abundance. It is worth noting that amino acids play a crucial role in the prevention of heart-related diseases. In the case of Bambara groundnuts, the presence of glutamic acid suggests its potential utilisation as a flavouring agent, while the presence of lysine acid implies that it can serve as an excellent complementary source of protein when added to cereals (Lin Tan et al., 2020).

#### **2.2.1.2 Carbohydrates**

Experts have highlighted the fact that carbohydrates are abundantly present in Bambara groundnuts and that consumption of Bambara groundnuts provides the highest amount of total energy when compared to other legumes like cowpeas and pigeon peas. A study conducted by Majola et al (2021) presents the above finding. Another study by Pahane et al (2017) reported a carbohydrate content range of 50-61.3%, while Hlanga et al (2021) found a higher percentage of 65%. Similarly, Majola et al. (2021) reported carbohydrate contents ranging from 51% to

71%. The differences may be influenced by factors such as geographic location and processing methods. In the context of legumes, carbohydrates are categorised as complex compounds due to their molecular structure and composition. It is worth noting that the carbohydrates found in Bambara groundnuts are not limited to a single type, but they encompass a variety of oligosaccharides and polysaccharides. These complex carbohydrates contribute significantly to the overall carbohydrate content of the Bambara groundnut (Khan et al., 2021). In fact, research conducted by Lin Tan et al (2020) and Tayade et al (2019) indicates that starch accounts for approximately 49.5% of the total carbohydrate content, while dietary fibre makes up around 10% to 20%. The dietary fibre found in Bambara groundnut plays a crucial role in promoting healthy digestion and managing cholesterol levels in the bloodstream (Martín-Cabrejas, 2019) which effectively prevents constipation and aids in maintaining optimal levels of blood cholesterol. Furthermore, Bambara groundnuts contain an insoluble starch (Tayade et al., 2019). It is primarily stored in the intestines of the human body and aids in the production of essential metabolites (Tayade et al., 2019). Furthermore, Bambara groundnut carbohydrates contain an insoluble fraction called starch, which is primarily stored in the intestines and serves as a precursor to produce essential metabolites within the human body (Tayade et al., 2019). This form of starch also functions as a bulking agent during the digestive process, enhancing overall digestion efficiency. Recent scientific research has shed light on the concept of resistant starch (RS), referring to the starch content that remains undigested (Lin Tan et al., 2020). It has been observed that RS has the potential to reduce the reliance on insulin in the human body, thereby offering promising implications for managing insulin levels (Lin Tan et al., 2020). The examination of starch content in various plant-based products has emerged as a significant area of investigation within the scientific community. This is primarily due to the diverse

applications of starch, including its use in the food industry for producing flour, as well as in non-food sectors such as paper manufacturing and the development of biodegradable plastics (Tayade et al., 2019).

### **2.2.1.3 Fat**

Margier et al (2018) made the ground-breaking discovery that legumes, while widely regarded for their nutritional value, contain a relatively low lipid content. Furthermore, it has been found that the fat content in Bambara groundnut, a specific type of legume, can vary significantly between different cultivars. According to the findings of Lin Tan et al (2020), the fat content in Bambara groundnut can range from as low as 1.4% to as high as 9.7%. Moreover, Majola et al (2021) reported an even higher fat content of 12% in their study conducted in 2021. These variations in fat content highlight the importance of considering the specific cultivar and its corresponding fat content when analysing the nutritional composition of Bambara groundnut. The differences may also be influenced by factors such as geographic location and processing methods. Interestingly, Lin Tan et al (2020) also found that the unsaturated fatty acids, particularly linoleic acid and oleic acid, are the predominant types of fatty acids present in Bambara groundnuts. The above-mentioned authors added that while these unsaturated fatty acids are generally considered beneficial for human health, it is important to note that they are susceptible to oxidation and rancidity. This susceptibility to oxidation and rancidity is a significant concern as it can negatively impact the quality and safety of Bambara groundnuts and their final products. Therefore, it is crucial to carefully consider and employ appropriate processing methods that can effectively reduce the potential effects of rancidity to ensure the production of high-quality Bambara groundnut products.

### **2.2.2 Mineral Composition**

All varieties of legumes have been documented in various studies as containing significant amounts of potassium, phosphorus, calcium, and magnesium, which are considered major minerals in their composition (Margier, et al., 2018). The research conducted by Lin Tan et al (2020) and Hlanga et al (2021) supports the notion that Bambara groundnuts are particularly rich in minerals, with potassium, magnesium, phosphorus, zinc, and iron being identified as the most abundant minerals within this legume variety. In a previous study conducted by Amarteifio, Tibe, & Njogu (2006), it was revealed that the macronutrient mineral levels (expressed in milligrams per 100 grams of dry matter) for Bambara groundnuts were as follows: calcium ranged from 37 to 128, potassium ranged from 1545 to 2200, magnesium ranged from 159 to 335, sodium ranged from 16 to 25, and Phosphorus ranged from 313 to 563. Additionally, the above-mentioned authors reported that the levels of micro minerals (expressed in parts per million) were reported as: copper ranged from 3.0 to 13.2, iron ranged from 23.0 to 150, and zinc ranged from 13.9 to 77.0. It is important to note that the mineral content of Bambara groundnuts can vary depending on the specific cultivar and may also be influenced by factors such as geographic location and processing methods. It is worth mentioning that the mineral levels found in Bambara groundnuts appear to be higher than those observed in chickpeas and mung beans, making this legume particularly advantageous in terms of its mineral composition (Lin Tan et al., 2020). However, it is crucial to consider the presence of anti-nutritional factors (ANFs) in Bambara groundnuts, as these compounds can have a negative impact on the bioavailability of the minerals present in this legume variety.

Table 3 Nutrient Composition of Bambara groundnuts (N'Dri Yao et al., 2015)

<b>Component</b>	<b>Composition g/100g fw</b>
<b>Proximate Composition</b>	
Moisture	11.7
Protein	18.8
Fat	1.4
Starch	50.2
Amylose	17.6
Sugars	2.4
Total Dietary Fibres	10.3
Soluble Fibre	0.5
Insoluble Fibre	9.8
Ash	2.9
<b>Minerals</b>	
Ca	30.2
Mg	136
P	335.8
Fe	8.8
Cu	0.5
Zn	1.9
Phytic acid	1.1

### **2.2.3 Anti-nutritional factors**

Although Bambara groundnuts have nutritional benefits, the presence of anti-nutritional factors (ANFs) poses a limitation. ANFs are constituents that hinder the optimal bioavailability or digestion of essential nutrients in the legume. The level of ANFs is believed to vary in the seeds based on their coat colour (Lin Tan et al., 2021). Prominent ANFs found in Bambara groundnuts include condensed tannins (CTs), trypsin inhibitors, and phytic acid (Lin Tan et al., 2021).

#### **2.2.3.1 Condensed Tannins**

Tannins are polyphenolic compounds that hinder the proteolytic activity and/or generate indigestible complexes with dietary proteins, thereby diminishing their digestibility (Yao et al., 2015). Legume-derived tannins, being water-soluble phenolic substances of substantial molecular weight, have the capability to form complexes that are not easily digestible (Adeleke et al., 2017). The formation of these complexes by tannins may result in a reduction in the availability of proteins (Adeleke et al., 2017). The seed coat generally contains tannins, and their elimination could effectively eliminate this toxic component, subsequently enhancing the nutritional value of food products (Adeleke et al., 2017). The concentration of tannins in Bambara groundnut has been reported to range between  $0.115 \pm 0.199$  mg g<sup>-1</sup>, although its levels vary across different seeds (Harris, Jideani, & Le Roes-Hill, 2018). Gqaleni (2014) and Tibe & Amarteifio (2007) have supported the notion that brown and red coloured seeds of Bambara groundnuts possess higher tannin content compared to the cream-coloured ones. Tannin compounds possess the capacity to confer bitterness and astringency to legumes, thereby influencing their palatability (Lin Tan et al., 2020). While elevated levels of CTs negatively impact protein digestibility, recent studies have discovered that low levels of tannins

are advantageous to both humans and animals as they serve as a preventive measure against the development of internal parasites (Unigwe et al., 2018). Remarkable quantities of tannins contribute to the reduction of blood glucose levels, offer protection against certain cancers, guard against diabetes, and reduce the risk of heart disease (Nwadi, Uchegbu & Okonkwo 2019).

### **3.2 Trypsin Inhibitors**

Trypsin inhibitors can be found in the raw seeds of legumes (Gemedede and Ratta, 2014). These inhibitors are specifically designed to hinder the activity of trypsin and chymotrypsin enzymes within the gastrointestinal tract (Tibe and Amarteifor, 2007) and Amarteifio, 2007; Unigwe et al., 2018). The subsequent inhibition of protease activity can have a negative impact on the digestion and absorption of proteins within the human body (Lin Tan et al., 2020). Reported trypsin levels in seeds can range from 5.30 to 73.40 TI mg/g, showing variation (Unigwe et al., 2018). Tibe, Amarteifio, and Njogu (2007) determined the composition of trypsin inhibitors to be within the range of 6.40 to 49.1 TI mg g<sup>-1</sup>. It is worth noting that raw, unprocessed seeds tend to contain higher levels of trypsin inhibitors compared to processed seeds.

#### **2.2.3.2 Phytic Acid**

Phytic acid is abundantly present in significant quantities within Bambara groundnuts (Meyes et al., 2019). The existence of elevated phytate levels is linked to the reduction in calcium accessibility (Gqaleni, 2014). It is well-known that the inhibition of gastrointestinal tyrosinase, trypsin, pepsin, lipase, and amylase activity is associated with the presence of phytate levels ranging from 1.35-4.93 mg/g in Bambara groundnuts (Unigwe et al., 2018). Phytic acid significantly affects mineral bioavailability in Bambara groundnut. Its presence can lead to

reduced absorption of essential minerals, particularly Iron, due to its ability to chelate with mineral cations and form insoluble complexes, making them less bio-accessible (Lin Tan, 2022). Although it is reported that phytic acid forms crosslinks with dietary proteins, starch, and digestive enzymes, thus negatively impacting the availability of nutrients, it is essential to recognize that phytic acid demonstrates antioxidant and anticancer traits, thereby suggesting its potential for promoting good health (Lin Tan et al., 2020).

Table 4 Nutritional composition of different BGN cultivars (Ramatsetse, Ramashia, & Mashau, 2023).

<b>Proximate composition (%)</b>					
<b>Cultivars</b>	<b>Protein</b>	<b>Fat</b>	<b>Moisture</b>	<b>Carbohydrates</b>	<b>Ash</b>
Red	19.5–20.57	5.2–6.5	8.0–8.7	54.4–63.9	2.4–3.4
Black	21.7–26.3	5.2–8.5	8.9–9.0	52.8–57.4	2.2–3.5
Cream	19.5–23.51	6.0–7.8	8.9–9.7	56.0–58.6	2.5–2.9
Brown	19.0–24.3	5.3–8.0	8.7–10.3	54.4–59.4	2.4–3.0

#### **2.2.4 Emulsifying properties**

The utilisation of proteins within the food industry holds significance due to its diverse range of functional properties. Functional properties that are crucial to the food industry include gelation, foaming, and emulsion capacities. These functional properties are fundamental physico-chemical characteristics that contribute to the performance of properties in food systems throughout processing, storage, and preparation (Eltayeb, Ali, Abou-Arab & Abu-Salem, 2011). The search for natural emulsifiers derived from plant or food products is particularly desirable owing to their non-toxic nature, affordability, availability, and established utilisation in food and medicine (Gabriel, Jideani & Ikhu-Omoregbe, 2013). Despite limited documented research indicating the potential of Bambara groundnut as a food-grade emulsifier, studies suggest that its combination of protein, starch, ash, and carbohydrate

make it a suitable emulsifier (Ijarotimi et al., 2009). Bambara groundnut proteins have the potential to be excellent ingredients for formulating food foams and emulsions (Arise, Nwachukwu, Aluko & Amonsou, 2017). Gabriel, Jideani, and Ikhu-Omoregbe (2013) supported the use of Bambara groundnut as a natural emulsifier in the food industry due to its ability to create stable oil-water emulsions. The emulsifying properties of this legume may also prove useful in various food products such as sausages, cake batters, and soups (Mubaiwa Fogliano, & Linnemann, 2016). Proteins isolated from legumes are a preferred raw material for beverages, infant formula, children's milk food, textured protein products, and specific types of specialty foods (Eltayeb, Ali, Abou-Arab & Abu-Salem, 2011). A study conducted by Huang et al (2018) indicated that the soaking of legumes enhances emulsifying activity and improves the structure and texture of gluten-free bread. Thus, suggest that various processing method impact the emulsifying property of Bambara groundnuts.

## **2.3 Rhizobium Inoculated Bambara Groundnut and its potential use in the food industry**

### **2.3.1 Background of inoculants in agriculture**

The soil in Namibia is distinguished by its deficient nitrogen content and scarcity of other essential trace elements (Sako, Mills, & Roychoudhury, 2009). This condition may be attributed to drought and substandard farming practices prevalent in the country. To illustrate this, in the northern regions of Namibia, the primary crops cultivated are pearl millet, maize, and sorghum (Embashu, & Nantanga, 2019). Farmlands cultivating such crops suffer from nitrogen deficiency. Consequently, the adoption of sound farming techniques like crop rotation and intercropping is scarce in Namibia. Soils lacking in nitrogen content led to diminished crop

production, which, in turn, exacerbates food insecurity. To tackle this issue, significant progress has been made in promoting the cultivation of leguminous crops.

The topic of legumes has been extensively studied on a global scale. In recent years, there has been a renewed interest in the cultivation of underutilized Bambara groundnut, cowpea, and soybean (Ouoba et al., 2019). Various underutilised legumes are believed to present opportunities for crop diversification and have the potential to make substantial contributions to food security and combat malnutrition (Soumare, Diedhiou, & Kane, 2021). Due to the protein and mineral richness of legumes like Bambara groundnut, they have the capacity to serve as an affordable substitute for protein for vulnerable communities that are unable to afford meat and fish (Ajilogba, Olanrewaju, & Babalola, 2022).

Bambara groundnuts possess microbial advantages due to their capability to fix nitrogen from the atmosphere (Fwanyanga, Horn, Sibanda & Reinhold-Hurek, 2022). Despite the global presence of numerous studies on the agronomical inoculation of Bambara groundnut, there remains a glaring absence of research specifically examining the impacts of processing methods on *Bradyrhizobium* inoculated Bambara groundnuts cultivated in Namibia. Furthermore, the effects of co-inoculation of other *rhizobacteria* that promote plant growth and strains of *Rhizobium* on leguminous plants have been scarcely reported in the literature (Nelwamondo, 2020). The limited utilisation of inoculated Bambara groundnut may also be attributed to a lack of understanding regarding the legume's utilisation and inherent value.

### **2.3.2 Nitrogen fixation in Bambara groundnut (*Vigna subterranean*)**

*Vigna subterranea* L. Verdc. belongs to the family *Leguminosae* (*Fabaceae*), specifically the subfamily *Faboideae* (Ibny et al., 2019). In addition to being a drought-resistant crop, plants belonging to the *Fabaceae* family can fix atmospheric nitrogen (Ibny et al., 2019). The soil

naturally contains *Rhizobia* bacteria, which are known to form a symbiotic relationship with Bambara groundnut through the development of nodulating nodules that fix atmospheric nitrogen (Lin Tan et al., 2020). This process involves a mutualistic association with the bacterium *Rhizobium*, which replaces soil nitrogen and enhances crop yields (Heller et al., 1997). Legumes also contribute organic sources such as carbon to the soil, providing a suitable environment for available *Rhizobia* (Nelwamondo, 2020). Leguminous crops have the capacity to biologically fix or accumulate nitrogen ranging from 14,500 to 90,800 kilograms per acre, thereby reducing the dependence on costly artificial nitrogen fertilisers (Andika, Abukutsa, Onyango, & Stutzel, 2014).

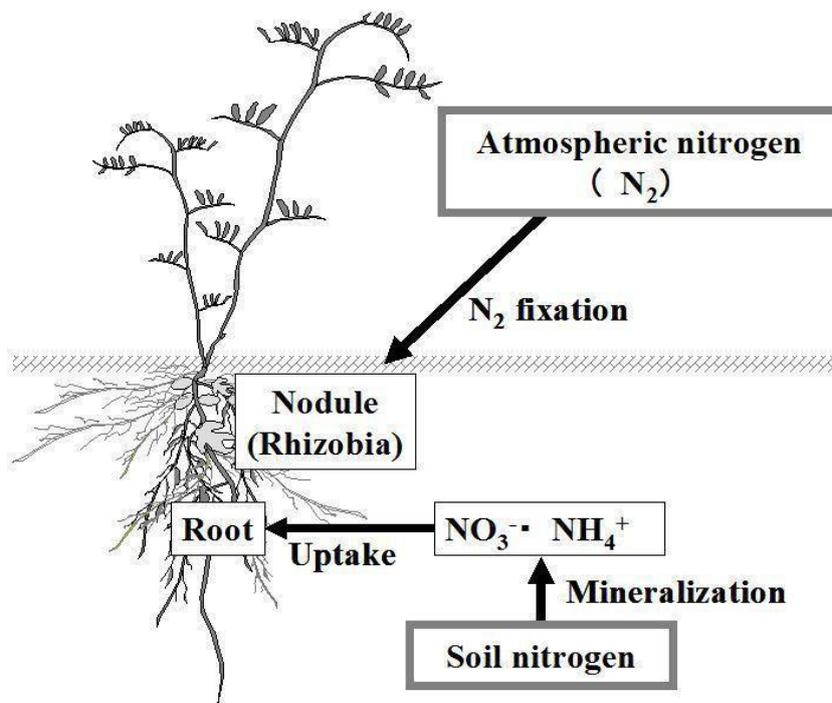


Figure 7 Biological process of nitrogen fixation (Sato, 2014)

### 2.3.3 Factors limiting biological nitrogen fixation in Bambara groundnut

The effectiveness of *Rhizobium* fixation in Bambara groundnuts is influenced by various factors including the geographic location, soil pH, temperature and moisture levels, soil

infertility, different agricultural techniques (such as crop rotation and intercropping), the application of organic fertilisers, and the use of microbial bio-fertilizers like *Rhizobia* and *Mycorrhiza* (Gomoung, Mbailao, Toukam, & Ngakou, 2017). A study conducted by Gomoung et al. (2107) demonstrated that the levels of soil nitrogen and phosphorus increased by 20.66% and 38.08% respectively after harvesting the inoculated Bambara groundnut. This observed rise has the potential to enhance both the soil quality and the yield of crops cultivated on the land. Moreover, the same study found that the yield of Bambara groundnut increased following the planting of inoculated legumes.

#### **2.3.4 The use of *Bradyrhizobium* in agriculture**

*Rhizobia*, traditionally categorised under the  $\alpha$ -subclass of Proteobacteria, encompasses several genera including *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Azorhizobium*, and *Methylobacterium* and recently discovered members of the genus *Burkholderia*, which belong to the  $\beta$ -proteobacteria group, as capable of forming nodules in legumes (Gerrano et al., 2021).

The utilisation of rhizobacteria holds significant importance in enhancing crop yield and safeguarding crops, particularly in legumes such as Bambara groundnut (Ajilogba, Olanrewaju, & Babalola, 2022). The nitrogen-fixing ability of legumes improves their capacity to be cultivated without the need for artificial nitrogen-fixing chemicals and fertilisers. Considering this, farmers who practise intercropping with Bambara groundnut and other crops would benefit greatly, as fertilisers can be costly and challenging to obtain. Moreover, Bambara groundnut can engage in cross-nodulation with nitrogen-fixing bacteria derived from other leguminous plants like cowpea, further intensifying its nitrogen-fixing capability (Ajilogba, Olanrewaju, & Babalola, 2022). This process may be described as a microbial-assisted

breeding technique aimed at achieving desirable production outcomes. By incorporating this approach into cropping systems involving other legume crops, a cost-effective and sustainable alternative to synthetic fertilisers in terms of nitrogen supply can be attained (Nelwamondo, 2020).

Among the diverse assemblage of plant bacteria, namely *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium*, there exists the potential for nitrogen fixation (Jaiswal, Mohammed, Ibny, & Dakora, 2021). These nitrogen-fixing bacteria have been documented to enhance the formation of soil structure, contribute to the decomposition of organic matter in the soil, play a significant role in the recycling of crucial elements, dissolve mineral nutrients, oversee the synthesis of various hormones responsible for regulating plant growth, decompose organic contaminants, stimulate the growth of healthy roots, and provide a natural means of controlling soil or seed-borne pathogens (Kenganyia, Anikwe, & Ngwu, 2017). The manifold advantages offered by plant growth-promoting bacteria can substantially diminish the necessity to employ synthetic chemical fertilisers. Information pertaining to the biodiversity of Rhizobia that nodulate the Bambara groundnut in African soils remains scarce; however, Ibny et al (2019) demonstrated that the Bambara groundnut has the capacity to fix approximately 4 to 200 kg N. Ha<sup>-1</sup>.

It is imperative that an effective strain of *Rhizobium* be utilised and that the nutritional requirements of the Bambara groundnut, aside from nitrogen, be adequately met, to fully exploit its nitrogen-fixing potential (Laurette et al., 2015). The practice of inoculating Bambara groundnuts with bacteria has been heralded to enhance food security; however, the knowledge in this domain remains largely untapped (Ajilogba, Olanrewaju, & Babalola, 2022; Gomoung, Mbailao, Toukam, & Ngakou, 2017).

The utilisation of synthetic fertilisers has the potential to induce soil acidification and experience reduced effectiveness over an extended period of production, resulting in an increased reliance on nitrogen fertilisers for optimal crop yield (Laurette et al., 2015). To address this issue, an alternative approach involves the utilisation of Phosphate solubilizing bacteria. Laurette et al., (2015) explained that the introduction of *Rhizobium* inoculants significantly enhances crop yield in various leguminous plants and can reduce the need for costly synthetic nitrogen fertilisers, which also have negative effects on soil properties.

As stated by Silva et al (2017), even though legumes are known for their high-quality proteins, they also contain a diverse range of bioactive compounds, including polysaccharides, bioactive peptides, phenolics (such as isoflavones), carotenoids, tocopherols, and fatty acids, among other phytochemicals. This makes legumes highly suitable as functional foods and potential candidates for incorporation into nutraceutical products. It is supported that the introduction of *Rhizobium* inoculants may have an impact on these bioactive compounds. To date there exists no study on the effects of *Bradyrhizobium* on the nutritional composition of Bambara groundnuts. *Bradyrhizobium* has the capability to increase the legume's capacity to acquire and employ nutrients and minerals. It has been observed through investigations that the introduction of *Bradyrhizobium* advanced the protein content of soybeans (Aklet et al., 2022). Similarly, another study conducted by Szpunar Krok et al (2021) revealed that the introduction of *Bradyrhizobium japonicum* increased the organic and fatty acid content of soybean seeds. However, Jarecki (2023) discovered in a separate study that the introduction of *Bradyrhizobium* strains diminished the crude fat content of the seeds. This implies that the impact *Bradyrhizobium* can have on the nutritional composition of Bambara groundnut can fluctuate

based on a range of factors, including the specific strain of *Bradyrhizobium*, the cultivar, and the growing conditions.

## **2.4 The effects of processing on Bambara groundnuts**

### **2.4.1 Background of processing Bambara groundnuts**

Bambara groundnut, which is a type of legume, is commonly cultivated by African communities. The processing of this crop, which is native to Africa, is primarily carried out using traditional techniques. Once harvested, the legume can undergo sun drying and subsequent storage to prolong its shelf life. When the seeds have reached a dry state, a pretreatment can be applied before initiating the cooking process (Obilana, & George, 2021). It is important to note that traditional processing methods in Namibia typically involve boiling and sun drying after harvest. However, other regions in Africa have introduced value-added techniques for this legume such as the production of cakes, porridge, soup, coffee substitutes, and even canning (Mubaiwa et al., 2016). It is crucial to acknowledge that various treatments have been identified to exert an impact on the nutritional, sensory, and functional properties of this legume. These findings align with a study conducted by Adeleke et al. (2017), which emphasised that processing methods not only affect the nutritional value of the Bambara groundnuts, but also have the potential to enhance their optimal utilisation. Variety of processing methods have been explored, including soaking, boiling, roasting, fermentation, germination, milling, and various combinations thereof, resulting in a diverse range of products (Mubaiwa et al., 2016).

### **2.4.2 Fermentation & Germination**

Fermentation & germination is a pre-treatment common and regularly practised amongst the *Ovambo* communities on *Pearl millet* and *Sorghum bicolor* [L.] *Moench* (Embashu, &

Nantanga, 2019). Unfortunately, there is no existing research on the effects of fermentation and germination specifically on Bambara groundnuts in Namibia. However, it is well-known that these methods can effectively break down non-digestible oligosaccharides, which are associated with the production of flatus, into simpler carbohydrates and enhance protein digestibility (Lin Tan et al., 2020). Moreover, by subjecting legumes to the processes of germination, fermentation, and roasting, the presence of anti-nutritional factors can be reduced to a tolerable level (Ijarotimi et al., 2009).

### **2.4.3 Soaking**

The practice of soaking Bambara groundnuts in either hot or cold water at varying time intervals may be carried out. Prolonged periods of immersion lead to a reduction in specific antinutrients present in the legume. Lin Tan et al (2020) reported that immersion generally has both positive and negative effects on the nutritional value of the seeds, primarily through the process of nutrients and ANFs leaching into the immersion water. The rate and extent of leaching are influenced by the binding strength of biomolecules to the intracellular matrix, which can be manipulated by the temperature and pH of the immersion liquid (Gaugiran et al., 2007). Studies have documented a greater loss of trypsin inhibitor and tannins in Bambara groundnut during immersion in hot water, whereas the opposite was observed for phytate (Lin Tan et al., 2020). Immersion also facilitates the subsequent processing of Bambara groundnut. Elevating the temperature of immersion (up to 60°C) enhances the rate of water absorption and the efficiency of dehulling, while reducing dehulling loss (Mubaiwa et al., 2018). This is due to the elevated temperature of the water potentially causing a weakening of the outer layer fibrous tissues of the legume (Lin Tan et al., 2020). With regards to functional properties, pre-

immersion of Bambara seeds prior to milling yields flour with increased foam capacity and improved pasting properties (Mubaiwa et al., 2018). From the perspective of food and nutritional security, immersion is a cost-effective and energy-efficient processing stage that can significantly enhance the utilisation of Bambara groundnut. However, the method of soaking may not be suitable at an industrial level as it may be costlier to manage bulk wastewater.

#### **2.4.4 Cooking aids**

The adverse effects of traditional post-harvesting methods on the hard to cook (HTC) phenomenon observed in legumes have gained widespread recognition. The susceptibility of beans to the occurrence of the HTC phenomenon increases significantly when legumes are stored at excessively high temperatures (above 25°C) and/or high levels of humidity (above 65%), as highlighted in the study by Lin Tan et al (2020). The exposure of legumes to extreme conditions of elevated temperature and humidity leads to a hardening process, ultimately resulting in the need for prolonged cooking durations and substantial energy consumption, as noted by Minka et al (1999). The occurrence of HTC in specific legumes is attributed to an inherited genetic condition, as explained by Lin Tan et al. (2020). It is crucial to acknowledge that HTC directly impacts the edibility and texture of legumes, as emphasised by Minka et al. (1999). In the case of HTC Bambara groundnuts, the boiling period is significantly longer compared to other legumes. Proper cooking of Bambara groundnuts requires approximately 4-5 hours, while soybeans require 3.6 hours, common beans 1.5 hours, cowpeas 2.4 hours, and mung beans 0.5 hours, as outlined in the study by Mubaiwa et al. (2017).

Moreover, Yahaya et al (2022) documented that the occurrence of HTC due to suboptimal storage conditions results in a reduction in the nutritional value of legumes. The above-

mentioned authors further explained that the consequences of HTC in Bambara groundnuts encompass a wide range of factors, which include diminished in vitro bioavailability of calcium and magnesium, impaired digestion of starch, reduced digestibility of protein, increased consumption of water and fuel during the cooking process leading to elevated cooking expenditures, especially in regions where fuel and firewood are not readily accessible. Additionally, HTC exacerbates the challenges associated with milling procedures in communities lacking appropriate milling equipment, as emphasised by Lin Tan et al (2020) and Mubaiwa et al (2017). It is important to note that the aforementioned factors contribute to the declining utilisation of Bambara groundnuts. While this may not pose a significant processing dilemma for communities equipped with advanced technological facilities, it undeniably presents a notable obstacle for rural communities in Namibia that heavily depend on traditional methods.

Various developmental changes occur in the hull, cell wall, cotyledon, and anti-nutrient content of legumes, contributing to the HTC phenomenon (Mubaiwa et al., 2019). The occurrence of HTC in legumes can be attributed to two mechanisms, namely the pectin-phytate and lignification mechanisms, both of which are a consequence of storage at elevated temperatures and humidity levels (Mubaiwa et al., 2017). Pectic substances, a significant component of the plant cell wall structure, require degradation to soften the internal cell tissue and, consequently, the bean itself (Tara, Christopher, & Peter, 2016). Phytic acid acts as a potent chelator for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions, hydrolysing into phytate-cation (Cheryan, & Rackis, 1980; Majumder, Datta, & Datta, 2019). Additionally, pectin methylesterase eliminates esters of pectin, resulting in the formation of free carboxyl groups that bind to free cations (Lin Tan et al., 2020). The continuous formation of these cations leads to the hardening of the legumes. Another well-

known hypothesis regarding HTC is cell lignification, which suggests that the degradation of proteins occurs between aromatic compounds and phenolic compounds, triggering the toughening of the cell wall (Mubaiwa et al., 2019). Alkaline salts reduce the cooking time of legumes by promoting the degradation of pectin compounds through ion exchange and chelation, whereby sodium ions replace or remove the internal cellular barriers (Tara, Christopher, & Peter, 2016).

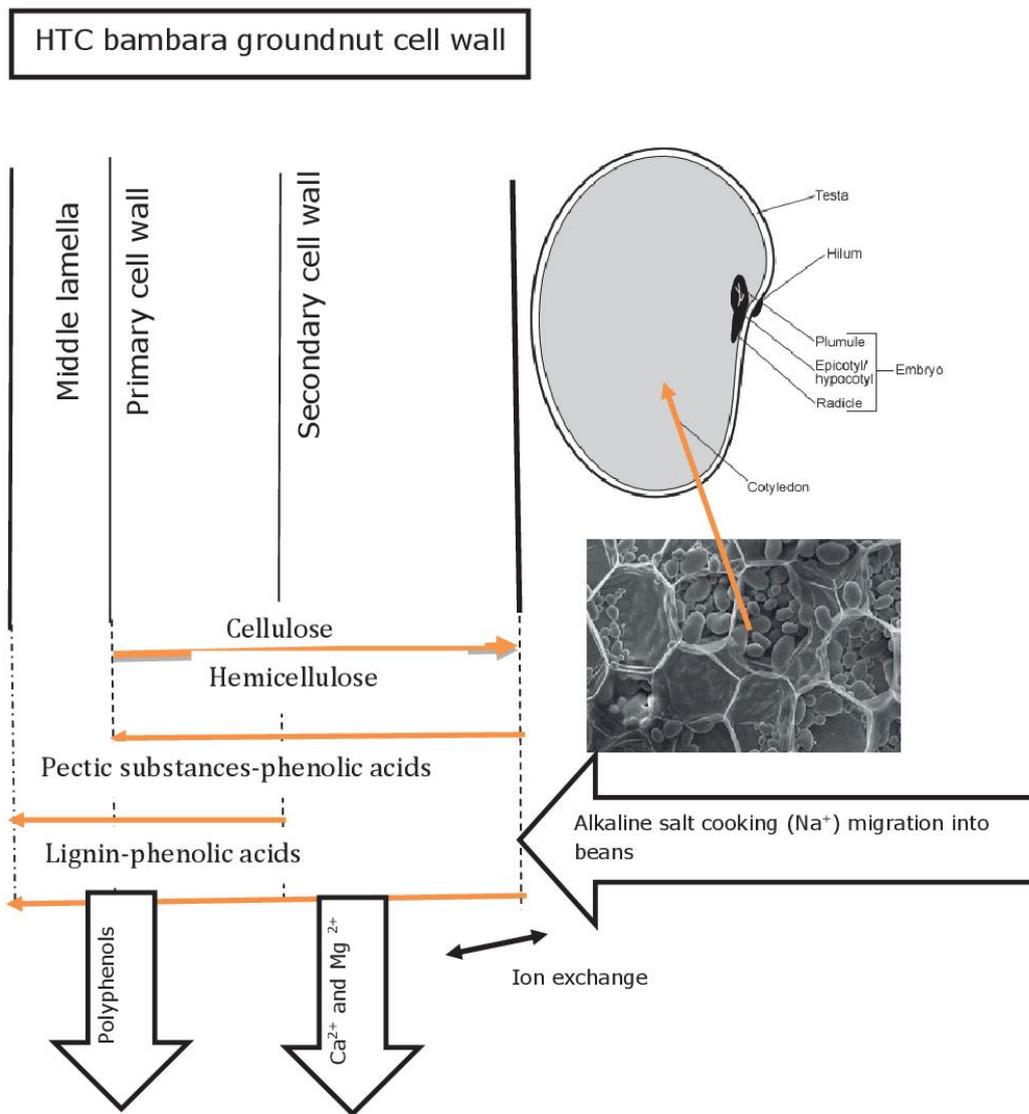


Figure 8 Development of HTC in Bambara groundnuts (Mubaiwa, 2018)

### **Chapter 3: Research Design**

This chapter presents the research design of the boiled and roasted inoculated and non-inoculated Bambara groundnut different cultivars. The research is divided into two parts. One part will highlight the research of boiling the inoculated and non-inoculated Bambara groundnut cultivars in different alkaline salts. The other part will highlight the research of roasting the inoculated and non-inoculated Bambara groundnut cultivars at different roasting times.

#### **3.1.1 Experimental design and treatments**

In this study, there are three independent variables;

- a) Inoculated and non-inoculated Bambara groundnuts
- b) Bambara groundnut cultivars with three factor levels (brown, cream and red)
- c) Processing methods with two factor levels (boiling and roasting)

The experimental design was a fully crossed factorial design with 12 factor level combinations. Each factor level combination was replicated two times. The research study was conducted at the University of Namibia, Neudamm Campus, Food Science and Systems Department.

#### **3.1.2 Research Ethics Consideration**

A research ethical clearance attached as appendix 1 was obtained from the University of Namibia through the Decentralized Ethics Committee.

### **3.2 Effect of *Bradyrhizobium* inoculation on the proximate, mineral, condensed tannins and emulsifying activity of Namibian grown Bambara groundnut flour**

#### **Abstract**

Bambara groundnut (*Vigna subterranea*) is as a source of carbohydrate, protein and minerals. Namibia is home to various Bambara groundnut cultivars. Although Bambara groundnut has the potential to address food security in Namibia, this legume remains underutilized. Bambara groundnut is a nitrogen fixing legume that forms symbiotic relationship with strains of *Rhizobia*. Inoculating Bambara groundnut with strains of *Rhizobia* has been discovered to improve plant yield and growth rate, however the nutritional composition of such legumes has not yet been studied. The aim of this study was to assess the proximate composition, mineral composition, condensed tannins (CT) and emulsifying activity (EA) of brown, cream and red *Bradyrhizobium* inoculated Bambara groundnut flours. The findings of the study indicate that there exists a statistically significant ( $P < 0.05$ ) between Bambara groundnuts that have been inoculated and non-inoculated. Bambara groundnuts inoculated with *Bradyrhizobium* had a higher protein, calcium, phosphorus, potassium, magnesium, phosphorus, sodium, copper, iron and manganese. However, all Bambara groundnuts cultivars inoculated Bambara groundnuts cultivars had a lower carbohydrate, fat and Zn. The emulsifying activity in inoculated Bambara groundnuts cultivars was reported highest. Condensed tannins could not be detected in non-inoculated and inoculated red Bambara groundnuts cultivars.

Keywords: Bambara groundnut (BGN); *Bradyrhizobium*; Emulsifying activity, Hard-to-cook phenomenon; Inoculation

### 3.2.1 Introduction

Bambara groundnut (*Vigna subterranean*) is a source of crude protein, carbohydrates, fats, minerals such as potassium, phosphorus, magnesium, zinc, iron (Lin Tan et al., 2020). Bambara groundnut protein is a potential emulsifying ingredient in the beverage industry (Alabi, Ali, Nwachukwu, Aluko, & Amonsou, 2020) and can act as an affordable protein substitute for vulnerable communities in Namibia that cannot afford meat and fish (Ajilogba, Olanrewaju, & Babalola, 2022). Bambara groundnuts which exist in varieties of cream, red, cream, black, purple and speckled seeds (Maphosa, Jideani, & Maphosa, 2022) are cultivated by communal farmers in the northern regions of Namibia (Fleissner, 1998).

Legumes are uniquely advantageous as they can atmospherically fix nitrogen through a symbiotic relationship with the bacterium *Bradyrhizobium*, thus increasing crop yields, protecting legumes from pests and reducing the need for artificial fertilisers (Heller et al., 1997). Inoculating Bambara groundnuts seeds with bacteria is described to improve food security however, knowledge in this area has been untapped (Ajilogba, Olanrewaju, & Babalola, 2022; Gomoung, Mbailao, Toukam, & Ngakou, 2017). Although several studies have been globally conducted on the agronomical benefits of co-inoculation of *Rhizobacteria* strains on Bambara groundnuts (Nelwamondo, 2020), no research has yet been carried out to particularly assess the inoculation effect on nutritional profile of Bambara groundnuts grown in Namibia. This study aimed to determine the effects of *Bradyrhizobium* inoculation on the proximate composition, mineral composition, emulsifying properties and condensed tannins of brown, cream and red Bambara groundnut cultivars grown in Namibia.

### **3.2.2 Source of material and equipment**

Bambara groundnuts not inoculated with *Bradyrhizobium* bacteria were purchased from Namibia Institute of Seeds (NIOS) in Windhoek, Namibia. *Bradyrhizobium* bacteria inoculated with Bambara groundnuts were harvested from a plantation from the University of Namibia.

### **3.2.3 Methods**

#### **3.2.3.1 Preparation of Bambara groundnut flour samples**

Bambara groundnuts (brown, cream and red) were blended into flour using a 1.8 L Hamilton Beach Food Blender.

### **3.2.4 Analysis**

#### **3.2.4.1 Proximate analysis of Bambara groundnut flour**

Proximate content (moisture, ash fat, crude protein, carbohydrates and crude fibre) of the Bambara groundnut flour were analysed following procedures of the Association of Official Analytical Chemists (AOAC International, 1995).

#### **3.2.4.2 Mineral Analysis of Bambara groundnut flour**

The mineral composition of Bambara groundnut flour was analysed by the AAS (Atomic Absorption Spectrophotometer) except for phosphorus that was analysed on a UVS (Ultraviolet Spectrometry). Elements were prepared using the dry ashing method, then acid digested using Hydro-chloric and Nitric Acids (AOAC International, 1990; Price, 1972).

#### **3.2.4.3 Emulsification activity of Bambara groundnut flour**

Emulsification activity (EA%) of Bambara groundnut flour was determined following the adjusted methods by Mubaiwa et al. (2018). Flour samples of 0.4 g, 20 mL sunflower oil (0.9 mL/g) and 20 mL of water (1.0 mL/g) which resulted in a 1% (w/v) mixture, were prepared. The samples were mixed for 1 minute using a vortex at 3000 rpm and centrifuged for 10

minutes at 1600 rpm. The height of the total content (TC) and the height of the emulsion layer (ELH) were measured. The EA was expressed using equation below:

$$EA\% = \frac{\text{Height of emulsion layer}}{\text{Height of Total Content}}$$

#### **3.2.4.4 Anti- Nutritional Factors**

Condensed tannins (CT) were analysed following the methods compiled by the University of Namibia, Food Science and Systems Department (Price, Van Scoyoc & Butler, 1978). Catechin stock solution was prepared by dissolving 0.01 g catechin into a 100 mL volumetric flask (100  $\mu\text{g}/\text{mL}$ ). Standard solutions of the concentration 0, 20, 40, 60, 80 and 100  $\mu\text{g}/\text{mL}$  in methanol were prepared. About 50  $\mu\text{L}$  of the sample extract, blank and standard was transferred into a 1 mL centrifuge tube. About 200  $\mu\text{L}$  vanillin reagent was added. The mixtures were mixed by vortex. The mixture was left in the dark for 15 minutes at room temperature (25-30°C). About 200  $\mu\text{L}$  of the reaction mixture was pipetted into a 96-well microplate. The absorbance was measured at 500 nm using a microplate reader (Gene sys 20, Thermo). From the readings provided, the blank was subtracted from the readings to eliminate non-tannin positives. The data was used to construct a calibration curve, the results were expressed as mg catechin/g sample (dry weight, DW).

#### **3.2.5 Data Analysis**

Results were recorded as mean  $\pm$  standard deviation of duplicate measurements. Data Analysis was done using IBM SPSS Statistics (version 29, 2022, IBM Inc., USA). Multivariate analysis of variance (MANOVA) was used to establish differences between treatments. Duncan's multiple range test was used to separate means where differences exist at 5% significance level.

### **3.2.6 Results and Discussions**

The main effects of inoculation ( $p = 0.001$ ) and cultivar ( $p = 0.026$ ) were significant on the emulsifying property of the flour. However, the effect of salt on the emulsifying property was not significant.

The effect of all the two-way interaction was significant ( $p = 0.001$ ) on the proximate composition of the flour. The two-way interaction effect of inoculation and cultivar ( $p = 0.001$ ) was significant on all the minerals except for calcium. The two-way interaction effect between the inoculation and cultivar was significant on emulsifying properties of the flour. The three-way interaction effect was significant ( $p = 0.001$ ) on the proximate and mineral composition of the flour. However, the three-way interaction effect on the emulsifying property of the flour was not significant.

The interaction effects estimated the combined effects of factors on the dependent variable. When an interaction effect is present, the impact of one factor depends on the level of the other factor. Hence, when interaction effects are present, interpretation of the main effects is incomplete. Since the independent variables have interaction effects on the proximate, mineral and emulsifying properties of the flour, it is interesting to understand how these effects depend on the treatment levels.

#### **3.2.6.1 Proximate composition of Bambara groundnut flour**

Details of the effect of inoculation based on cultivar on the proximate composition of Bambara groundnuts flour are presented in Table 3. Inoculation produced significant differences in moisture ( $p = 0.001$ ), fat ( $p = 0.0001$ ) and crude fibre ( $p = 0.0001$ ) of Bambara groundnuts flour. Inoculation on brown Bambara groundnuts cultivar was not significantly different in ash ( $p =$

0.805), protein ( $p = 0.157$ ) and carbohydrate ( $p = 303$ ) while a significant difference was observed in moisture ( $p = 0.0001$ ), fat ( $p = 0.0001$ ) and crude fibre ( $p = 0.0001$ ). Additionally, inoculation of cream Bambara groundnuts cultivar was not significantly different on moisture ( $p = 0.794$ ), fat ( $p = 0.700$ ) and crude fibre ( $p = 0.247$ ) while a significant effect was observed in ash ( $p = 0.001$ ), protein ( $p = 0.001$ ) and carbohydrate ( $p = 0.008$ ). Inoculation of red Bambara groundnuts cultivar was not significantly different on moisture ( $P = 0.840$ ) and crude fibre ( $p = 0.193$ ) while a significant difference was observed in ash ( $p = 0.001$ ), fat ( $p = 0.001$ ), protein ( $p = 0.0001$ ) and carbohydrate ( $p = 0.0001$ ). The above results basically mean that there is a relationship between cultivar and treatment and as a result the cultivars do have an effect on the inoculation treatment.

Table 5 Effect of *Bradyrhizobium* inoculation on the proximate composition of Bambara groundnut cultivars

Proximate (%)	Moisture		Ash		Fat		Protein		Carbohydrate		Crude fibre	
	<i>Bradyrhizobium</i> Treatment		NO	IN	NO	IN	NO	IN	NO	IN	NO	IN
<b>Brown BGN</b>	6.57 ± 0.05 <sup>a</sup>	6.36 ± 0.09 <sup>a</sup>	2.54 ± 0.05 <sup>a</sup>	2.58 ± 0.35 <sup>b</sup>	6.47 ± 0.02 <sup>a</sup>	5.40 ± 0.21 <sup>b</sup>	19.44 ± 2.99 <sup>a</sup>	22.90 ± 4.66 <sup>a</sup>	64.98 ± 2.90 <sup>b</sup>	62.60 ± 4.52 <sup>a</sup>	5.60 ± 0.66 <sup>b</sup>	7.86 ± 0.78 <sup>a</sup>
<b>BGN Cultivars</b>												
<b>Cream BGN</b>	6.22 ± 1.74 <sup>a</sup>	6.42 ± 0.56 <sup>a</sup>	2.54 ± 0.08 <sup>a</sup>	3.12 ± 0.29 <sup>b</sup>	6.65 ± 0.60 <sup>b</sup>	6.55 ± 0.22 <sup>a</sup>	13.22 ± 3.20 <sup>b</sup>	19.42 ± 0.39 <sup>b</sup>	71.37 ± 4.88 <sup>a</sup>	64.66 ± 1.13 <sup>a</sup>	6.86 ± 0.93 <sup>a, b</sup>	7.87 ± 1.80 <sup>a</sup>
<b>Red BGN</b>	6.17 ± 1.19 <sup>a</sup>	6.32 ± 1.35 <sup>a</sup>	2.60 ± 0.06 <sup>a</sup>	4.05 ± 0.71 <sup>a</sup>	7.26 ± 0.40 <sup>a</sup>	6.47 ± 0.16 <sup>a</sup>	8.74 ± 1.88 <sup>a</sup>	19.40 ± 0.59 <sup>a</sup>	75.24 ± 3.26 <sup>a</sup>	63.77 ± 1.93 <sup>a</sup>	7.37 ± 1.64 <sup>a</sup>	8.31 ± 0.20 <sup>a</sup>

Results are expressed as means ± standard deviation of duplicates. Values with different subsets in the rows differ significantly. NO: BGN cultivars not inoculated with *Bradyrhizobium*, IN: BGN cultivars inoculated with *Bradyrhizobium*. BGN: Bambara groundnuts

The effect of *Bradyrhizobium* inoculation on the proximate composition of BGN cultivars is reported in table 3. According to Silva et al (2017) inoculating legumes with *Bradyrhizobium* bacteria causes metabolic and nutritional changes in the plant, leaves and seeds. *Bradyrhizobium* inoculated BGN cultivars all had a higher ash content. Red BGN inoculated with *Bradyrhizobium* had the highest ash content (4.05%). The higher ash content in inoculated Bambara groundnut agrees with results obtained by Elsheikh, & Elzidany (1997).

Results indicate that inoculated Bambara groundnut cultivars all had a lower carbohydrate content as compared to non-inoculated BGN. Reports have confirmed that the high concentration of nitrogen available to the plant which increases the protein content in inoculated plants subsequently reduces the carbohydrate levels (Elsheikh, & Elzidany 1997; Zahran, 1999). Red Bambara groundnut not inoculated with *Bradyrhizobium* had the highest fat (7.26%). Results from this study indicate that inoculated Bambara groundnut cultivars all had a lower fat content. Red Bambara groundnut not inoculated with *Bradyrhizobium* had the highest carbohydrate (75.24%). A study by Silva et al (2013) indicated that soybeans that were inoculated with *Bradyrhizobium* contained a higher total fatty acid content due to the increase in the monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) contents (DiNicolantonio, & O’Keefe, 2022). These findings do not agree with the results from this study. The reason behind low fat content is not clear but may be attributed to genetic and geographical origins of Bambara groundnut. Legumes grown in poor quality soils such as the ones known in Namibia may subsequently have a lower fat content (Smith, Reis Hodecker, Fuentes & Merchant, 2022).

Results from this study indicate that the protein content of inoculated Bambara groundnut and non-inoculated Bambara groundnut is not significantly different except in red varieties. This

difference is a result of host-specific nature of symbiotic relationship that causes an interaction between the Bradyrhizobium strains and various elements (Zn, N, K and P) in red BGN (Puozaa, Jaiswal, & Dakora, 2019; Liu, et al., 2015). Brown Bambara groundnut inoculated with *Bradyrhizobium* had the highest protein (22.90%). A study by Farfour, Al-Saman, & Hamouda (2015) showed that faba beans (*Vicia faba*) inoculated with *Rhizobium leguminosarum symbiovar viciae* had a notable increase in the proteins significantly. These results are similar and agree with results in this study. Numerous studies have documented that the introduction of various types and species of *Rhizobia* into several types of grain legumes, including soybean, cowpea, chickpea, faba bean, and peanut, led to enhancements in the protein composition (Silva et al., 2017).

#### **3.2.6.2 Mineral composition of Bambara groundnut flour**

From the two way interaction of cultivar effect on inoculation treatment, the effects of cultivar on the treatment of mineral composition are explained below here. Inoculation on brown Bambara groundnut cultivar was significantly different in Mg ( $p = 0.0001$ ), P ( $p = 0.0001$ ), Mg ( $p = 0.001$ ), Cu ( $p = 0.011$ ), Mn ( $0.0001$ ) and Zn ( $p = 0.0001$ ) while no significant effect was observed in K ( $p = 0.075$ ), Na ( $p = 0.163$ ) and Fe ( $p = 0.644$ ). Inoculation of cream BGN cultivar was significantly different in Ca ( $p = 0.0001$ ), P ( $p = 0.0001$ ), K ( $p = 0.003$ ), Na ( $p = 0.499$ ), Cu ( $p = 0.0001$ ), Fe ( $p = 0.0001$ ), Mn ( $p = 0.008$ ) and Zn ( $p = 0.007$ ) while no significant effect was observed in Mg ( $p = 0.974$ ). Inoculation of red Bambara groundnut cultivar was significantly different in Ca ( $p = 0.0001$ ), P ( $p = 0.001$ ), Mg ( $p = 0.046$ ), K ( $p = 0.0001$ ), Cu ( $p = 0.0001$ ), Mn ( $p = 0.004$ ) and Zn ( $p = 0.004$ ) while no significant effect was observed in Na ( $p = 0.096$ ) and Fe ( $p = 0.764$ ). The above results basically mean that there is a relationship

between cultivar and treatment and as a result the cultivars do have an effect on the inoculation treatment.

Table 6 Effect of *Bradyrhizobium* inoculation on the mineral composition of Bambara groundnut cultivars

Minerals	<i>Bradyrhizobium</i> Treatment	BGN Cultivar		
		Brown BGN	Cream BGN	Red BGN
Ca	NO	0.02 ± 0.00 <sup>a</sup>	0.02 ± 0.00 <sup>b</sup>	0.01 ± 0.00 <sup>c</sup>
	IN	0.03 ± 0.00 <sup>a</sup>	0.03 ± 0.0 <sup>a</sup>	0.03 ± 0.00 <sup>a</sup>
P	NO	0.20 ± 0.03 <sup>a</sup>	0.17 ± 0.02 <sup>b</sup>	0.21 ± 0.02 <sup>a</sup>
	IN	0.33 ± 0.01 <sup>a</sup>	0.32 ± 0.01 <sup>a</sup>	0.28 ± 0.03 <sup>b</sup>
Mg	NO	0.12 ± 0.00 <sup>a</sup>	0.12 ± 0.01 <sup>a</sup>	0.12 ± 0.01 <sup>a</sup>
	IN	0.11 ± 0.00 <sup>b</sup>	0.12 ± 0.01 <sup>b</sup>	0.17 ± 0.05 <sup>a</sup>
K	NO	0.02 ± 0.00 <sup>b</sup>	0.02 ± 0.00 <sup>b</sup>	0.03 ± 0.00 <sup>a</sup>
	IN	0.02 ± 0.00 <sup>b</sup>	0.02 ± 0.00 <sup>c</sup>	0.04 ± 0.00 <sup>a</sup>
Na	NO	0.03 ± 0.02 <sup>a</sup>	0.06 ± 0.08 <sup>a</sup>	0.01 ± 0.01 <sup>a</sup>
	IN	0.04 ± 0.01 <sup>a</sup>	0.03 ± 0.02 <sup>a</sup>	0.15 ± 0.19 <sup>a</sup>
Cu	NO	8.27 ± 1.46 <sup>a</sup>	3.52 ± 0.50 <sup>b</sup>	4.13 ± 1.23 <sup>b</sup>
	IN	6.41 ± 0.10 <sup>b</sup>	6.47 ± 1.00 <sup>b</sup>	8.77 ± 0.42 <sup>a</sup>
Fe	NO	34.07 ± 2.53 <sup>a</sup>	19.78 ± 0.39 <sup>b</sup>	35.40 ± 3.91 <sup>a</sup>
	IN	32.56 ± 7.31 <sup>a</sup>	28.48 ± 0.85 <sup>a</sup>	33.54 ± 14.24 <sup>a</sup>
Mn	NO	15.63 ± 1.30 <sup>c</sup>	23.07 ± 0.72 <sup>b</sup>	29.15 ± 0.47 <sup>a</sup>
	IN	24.68 ± 1.04 <sup>a</sup>	26.40 ± 1.98 <sup>a</sup>	18.85 ± 6.70 <sup>b</sup>
Zn	NO	20.60 ± 2.00 <sup>b</sup>	16.00 ± 0.30 <sup>c</sup>	25.25 ± 4.35 <sup>a</sup>
	IN	15.62 ± 1.53 <sup>b</sup>	16.74 ± 0.46 <sup>a</sup>	17.70 ± 2.27 <sup>a</sup>

*Results are expressed as means ± standard deviation of duplicates. Values with different subsets in the columns differ significantly.*

*NO: BGN cultivars not inoculated with Bradyrhizobium, IN: BGN cultivars inoculated with Bradyrhizobium.*

*Ca, P, Mg, K, Na (expressed in %)*

*Cu, Fe, Mn, Zn (expressed in mg/Kg)*

*BGN: Bambara groundnut*

The effect of *Bradyrhizobium* inoculation on the mineral composition of Bambara groundnut cultivars is reported in table 6. Results show that Bambara groundnut inoculated with *Bradyrhizobium* have a higher calcium, phosphorus, potassium, magnesium, sodium, copper, iron and manganese. Cream Bambara groundnut has a lower polyphenol content (Okafor, Jideani, & Meyer, 2022). Polyphenolic compounds in living organisms were found to reduce the concentration of minerals such as zinc (Hussain et al., 2020). Brown, cream and red Bambara groundnut inoculated with *Bradyrhizobium* had the highest phosphorus (0.28 - 0.33%). Red BGN inoculated with *Bradyrhizobium* had the highest magnesium (0.17%), copper (8.77 mg/Kg) and iron (33.54 mg/Kg). According to Mubaiwa et al (2016) red varieties of Bambara groundnut have been reported to contain an iron level twice as much as those found in cream beans. Several studies indicated other mineral compositions of Bambara groundnut; however, the zinc levels were not specified (Chelangat, Muturi, Gichimu, Gitari, & Mukono, 2023). Red BGN not inoculated with *Bradyrhizobium* had the highest manganese (29.15 mg/Kg) and zinc (25.25 mg/Kg). According to Mandizvo, & Odindo (2019), darker coloured Bambara groundnut was found to contain a higher mineral composition than those of lighter coloured Bambara groundnut. Furthermore, a study on inoculating Bambara groundnut with bacteria found that darker coloured seed Bambara groundnut was found to have a better mineral absorption ability as compared to lighter seed coat coloured Bambara groundnut (Zondi, 2012). The existence of a specific strain of bacteria such as *Bradyrhizobium* can enhance the bioavailability of minerals in legumes by reducing the quantity of antinutrients, such as phytic acid, which bind to minerals and diminish their absorption (Nkhata, Ayua, Kamau, & Shingiro, 2018; Knez, Kadac-Czapska, & Grembecka, 2023). This is further supported by findings by Makoi, Bambara, & Ndakidemi, (2013) that show that inoculating common beans (*Phaseolus*

*vulgaris* L.) with *rhizobium* bacteria significantly improved the uptake of minerals such as phosphorus, potassium, calcium, and manganese in different plant parts such as leaves, shoots, roots, and pods. However, the zinc levels of inoculated Bambara groundnut were lower. Studies suggest that *Rhizobia* and *Bradyrhizobium* possess the ability to regulate their symbiotic relationship through the utilisation of zinc starvation mechanisms (Zhang et al., 2020). The uptake of zinc during *Rhizobial* interactions with legumes is crucial during both the nodulation and nitrogen-fixation stages (Zhang et al., 2020). ANFs which are common in darker coloured Bambara groundnut are known to reduce zinc (Lin Tan et al., 2023). However, there is a lack of comprehensive understanding regarding the impacts of *Rhizobia* on zinc within legumes. This suggests that more effective processing methods are needed to increase zinc release from dark coloured Bambara groundnut.

### **3.2.6.3 Emulsifying Activity of Bambara groundnut flour**

From the two way interaction of cultivar effect on inoculation treatment, the effects of cultivar on the treatment of the emulsifying activity are explained below here. Inoculation on brown ( $p = 0.0001$ ), cream ( $p = 0.007$ ) and red ( $p = 0.0001$ ) Bambara groundnut cultivars were significantly different on the emulsifying activity. The above results basically mean that there is no relationship between cultivar and treatment and as a result the cultivars do not have an effect on the inoculation treatment.

Table 7 Effect of *Bradyrhizobium* inoculation on the emulsifying activity of Bambara groundnut cultivars

<i>Bradyrhizobium</i> Treatment	BGN Cultivar		
	Brown BGN	Cream BGN	Red BGN
NO	39.54 ± 2.38 <sup>a</sup>	39.73 ± 17.30 <sup>a</sup>	41.22 ± 1.56 <sup>a</sup>
IN	48.84 ± 0.66 <sup>b</sup>	63.96 ± 1.15 <sup>a</sup>	48.84 ± 1.37 <sup>b</sup>

*Results are expressed as means ± standard deviation of duplicates. Values with different subsets in the columns differ significantly. Results are expressed in %. NO: BGN cultivars not inoculated with Bradyrhizobium, IN: BGN cultivars inoculated with Bradyrhizobium.*

*BGN: Bambara groundnut*

The effect of *Bradyrhizobium* inoculation on the emulsifying activity (EA) of Bambara groundnut cultivars is reported in table 5. Results show that inoculated Bambara groundnut have a higher emulsifying activity. Cream Bambara groundnut inoculated with *Bradyrhizobium* had the highest emulsifying activity (63.96%). Red and brown Bambara groundnut are darker in colour and are known to contain higher polyphenols (Okafor, Jideani, & Meyer, 2022). A high polyphenol content has been found to reduce emulsifying properties and emulsifying activity (Li et al., 2022). This explains why cream Bambara groundnut had a higher emulsifying activity as compared to brown and red Bambara groundnut. A higher emulsifying activity in inoculated Bambara groundnut suggests that that inoculation may serve as a good emulsifier. A good emulsifying property means that inoculated Bambara groundnut can be used as an emulsifier in meat products (minced meat, sausages, ham), starch ingredient, salad dressing, ice-cream, mayonnaise and beverages (Zhang et al., 2023).

### 3.2.6.4 Condensed tannins of Bambara groundnut flour

Table 8 Effect of *Bradyrhizobium* inoculation on the condensed tannins of Bambara groundnut cultivars

Bradyrhizobium Treatment	BGN Cultivar		
	Brown BGN	Cream BGN	Red BGN
Non-inoculated	0.74	0.17	ND
Inoculated	ND	0.40	ND

*Results are expressed as means of duplicates. Values with different subsets in the columns differ significantly. Results are expressed in mg/g. ND = Not Detected. NO: BGN cultivars not inoculated with Bradyrhizobium, IN: BGN cultivars inoculated with Bradyrhizobium. BGN: Bambara groundnut*

The effect of *Bradyrhizobium* inoculation on the condensed tannins of Bambara groundnut cultivars is reported in table 6. Results show that non-inoculated brown Bambara groundnut had the highest concentration (0.74 mg/g) of condensed tannins while non-inoculated cream Bambara groundnut had the lowest (0.17 mg/g) condensed tannins. The reason behind inability to detect condensed tannins is as follows. Condensed tannins may be present in Bambara groundnut but in extremely low concentrations that are not easily detectable (Harris, Jideani, & Rose-Hill, 2018). The presence of other ANFs and flavouring compounds may be too strong that they mask the astringency of condensed tannins making them less detectable (Harris, Jideani, & Rose-Hill, 2018). The structure of condensed tannins differs in Bambara groundnut cultivars and can form complexes with proteins which can reduce their ability to be detected (Chung et al., 1998).

### 3.2.7 Conclusion

In conclusion, inoculated Bambara groundnut cultivars had a relatively higher protein but lower fat. Red Bambara groundnut inoculated with *Bradyrhizobium* had a higher magnesium, iron and copper. Cream inoculated with *Bradyrhizobium* had a higher emulsifying activity. Although a two-week training in 2022 was provided to students by Professor Percy Chimwamurombe from the Namibia University of Science and Technology in Namibia on inoculation of BGN grown in Namibia, there is still a great need to educate the community and small-scale farmers on the agronomical and nutritional benefits of inoculants. As highlighted by Fwanyangwa et al (2022) it is cheaper to inoculate Bambara groundnut with *Bradyrhizobia* bacteria as compared to other strains. Therefore, it is imperative to encourage policy makers, the Ministry of Agriculture, Water and Forestry and the Namibian Agronomic Board to support the use of inoculants. This will serve as a cheaper advantage as the current prices of fertilisers in Namibia are ever increasing. With the information provided in this study, product developers are encouraged to utilise Bambara groundnut to manufacture new food products that will act as meat replacers, vegan options or fortified products for school-going children. There is still a need to explore processing methods that will be suitable in releasing the perceived trapped nutrients from the intracellular structure of Bambara groundnut cultivars.

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### **3.3 Effect of roasting at different times on the proximate, mineral and emulsifying activity of Namibian grown *Bradyrhizobium* inoculated Bambara groundnut flour**

#### **Abstract**

Roasting is regarded as a dry heating method used to pretreat Bambara groundnuts. Bambara groundnut is as a source of carbohydrate, protein and various minerals. This study involved subjecting Bambara groundnut inoculated and not inoculated with *Bradyrhizobium* to roasting for 15, 30 and 45 minutes and grinding to yield flour. The resulting Bambara groundnut flours were then subjected to analysis encompassing proximate analysis, mineral composition and emulsifying activity (EA). The findings of the study indicate that there exists a statistically significant ( $P < 0.05$ ) between Bambara groundnut that have been roasted at different times. Results indicate that an increase in roasting time reduced certain nutritional composition while others increased with an increase in roasting time. There is a need to further research how seed coat colour interacts with the roasting time to affect the overall nutrition of Bambara groundnut.

Keywords: ANFs, Bambara groundnut (BGN), *Bradyrhizobium*; Inoculated, Roasting time

### 3.3.1 Introduction

Bambara groundnut, which is both nutritious and drought-tolerant, has the potential to contribute significantly to the Sustainable Development Goals (SDGs) established by the United Nations. By offering a cost-effective source of protein and essential nutrients to subsistence farmers and rural communities, including Namibia, it can play a role in reducing poverty (Lin Tan et al., 2020). Moreover, Bambara groundnut can serve as a sustainable and economical source of complex carbohydrates, plant-based protein, unsaturated fatty acids, as well as vital minerals like magnesium, iron, zinc, and potassium thereby contributing to global food security and nutrition (Lin Tan et al., 2020). Recognized for its high nutritional value, Bambara groundnut can address issues of malnutrition and food insecurity in Africa (Hlanga, Modi, & Mathew, 2021). This crop contains numerous biologically active phytochemicals that function as antioxidants, thereby contributing to overall health and well-being (Okafor, Jideani, Meyer, & Roses-Hill, 2022).

*Rhizobium* inoculation of legumes is a vital process that enhances crop productivity and nitrogen fixation. Specific *rhizobia* species form nodules on legume species, each requiring a specific strain. Several works reported that the inoculation of different genera and species of *rhizobia* in several grain legumes, such as soybean, cowpea, chickpea, faba bean, or peanut, produced increases in the nutritional content. Increased nutritional composition of Bambara groundnut is suggested by *rhizobia* (Fwanyanga et al., 2022). However, there is a knowledge gap in the seed system, agronomic practices, processing, and utilisation of Bambara groundnut. Within the communal farming areas of the northern regions of Namibia, Bambara groundnut holds the status of being the second most important legume, following cowpea (Fleißner, 2006). The estimated total production area of Bambara groundnut in Namibia is approximately

3000 hectares (Fleißner, 2006). Different varieties of Bambara groundnut exist, including cream with brown-eye, cream with black-eye, red, cream with no eye, black, purple, and speckled (Mamphosa, Jideani, & Mamphosa, 2022). However, the utilisation of Bambara groundnut is hindered by various factors, including a lack of knowledge regarding improved seed systems, agronomic practices, processing, and utilisation (Lin Tan et al., 2020). Traditional processing methods, such as dehulling and roasting, may impact the nutritional content, processing techniques, and overall quality of the crop (Lin Tan et al., 2020).

Roasting is classified as a processing method that utilises dry heat. Roasting is regarded as a low capital requiring method that requires less capital or resources. According to Ndidi et al (2014) different processing methods affect the bioavailability of nutrients in Bambara groundnut. This is because different nutrients are either lost or released during processing. In a study recorded by Ndidi et al (2014) reported that the tannin, trypsin inhibitor, calcium, potassium, and phosphorus levels were significantly reduced when Bambara groundnuts were subjected to a roasting of 300°C for 1 hour.

This may suggest that the time temperature effect may directly affect the nutritional content of the legume. The aim of this study was to determine the effects of roasting inoculated and non-inoculated Bambara groundnuts at different times, namely 15, 30 and 45 minutes at 140°C.

### **3.3.2 Source of material and equipment**

Bambara groundnuts not inoculated with *Bradyrhizobium* bacteria were purchased from Namibia Institute of Seeds (NIOS) in Windhoek, Namibia. *Bradyrhizobium* bacteria inoculated with Bambara groundnut were harvested from a plantation from the University of Namibia.

### **3.3.3 Methods**

#### **3.3.1 Preparation of Bambara groundnut flour samples**

Bambara groundnuts (brown, cream and red) were roasted in the oven at 140°C for 15, 30 and 45 minutes respectively. The beans were blended into flour using a 1.8 L Hamilton Beach Food Blender. The process applied was adjusted and followed from the similar research done by Ijarotimi et al (2009).

#### **3.3.4 Analysis**

##### **3.3.4.1 Proximate analysis of Bambara groundnut flour**

Proximate content (moisture, ash fat, crude protein, carbohydrates and crude fibre) of the Bambara groundnut flour were analysed following procedures of the Association of Official Analytical Chemists (AOAC International, 1995).

##### **3.3.4.2 Mineral Analysis of Bambara groundnut flour**

The mineral composition of Bambara groundnut flour was analysed by the AAS (Atomic Absorption Spectrophotometer) except for phosphorus that was analysed on a UVS (Ultraviolet Spectrometry). Elements were prepared using the dry ashing method, then acid digested using Hydro-chloric and Nitric Acids (AOAC International, 1990; Price, 1972).

##### **3.3.4.3 Emulsification activity of Bambara groundnut flour**

Emulsification activity (EA%) of Bambara groundnut flour was determined following the adjusted methods by Mubaiwa et al. (2018). Flour samples of 0.4 g, 20 mL sunflower oil (0.9 mL/g) and 20 mL of water (1.0 mL/g) which resulted in a 1% (w/v) mixture, were prepared.

The samples were mixed for 1 minute using a vortex at 3000 rpm and centrifuged for 10 minutes at 1600 rpm. The height of the total content (TC) and the height of the emulsion layer (ELH) were measured. The EA was expressed using equation 1:

$$EA\% = \frac{\text{Height of emulsion layer}}{\text{Height of Total Content}}$$

### **3.3.5 Data Analysis**

Results were recorded as mean  $\pm$  standard deviation of duplicate measurements. Data Analysis was done using IBM SPSS Statistics (version 29, 2022, IBM Inc., USA). Multivariate analysis of variance (MANOVA) was used to establish differences between treatments. Duncan's multiple range test was used to separate means where differences exist at 5% significance level.

### **3.3.6 Results and Discussions**

#### **3.3.6.1 Proximate composition of Bambara groundnut flour**

From the two way interaction of inoculation treatment and time treatment, the effects of inoculation on the time treatment of the proximate composition are explained below here. Non inoculation on time treatment of Bambara groundnut was not significantly different on moisture ( $p = 0.844$ ), ash ( $p = 0.607$ ), fat ( $p = 0.211$ ) and crude fibre ( $p = 0.053$ ) while it was significantly different in protein ( $p = 0.0001$ ) and carbohydrate ( $p = 0.0001$ ). Inoculation on time treatment of Bambara groundnut was not significantly different in ash ( $p = 0.077$ ), protein ( $p = 0.841$ ), carbohydrate ( $p = 0.428$ ) and crude fibre ( $p = 0.163$ ) while a significant effect was observed in moisture ( $p = 0.049$ ) and fat ( $p = 0.037$ ). The above results basically mean that there

is a relationship between inoculation and time treatment and as a result the inoculation does have an effect on time treatment.

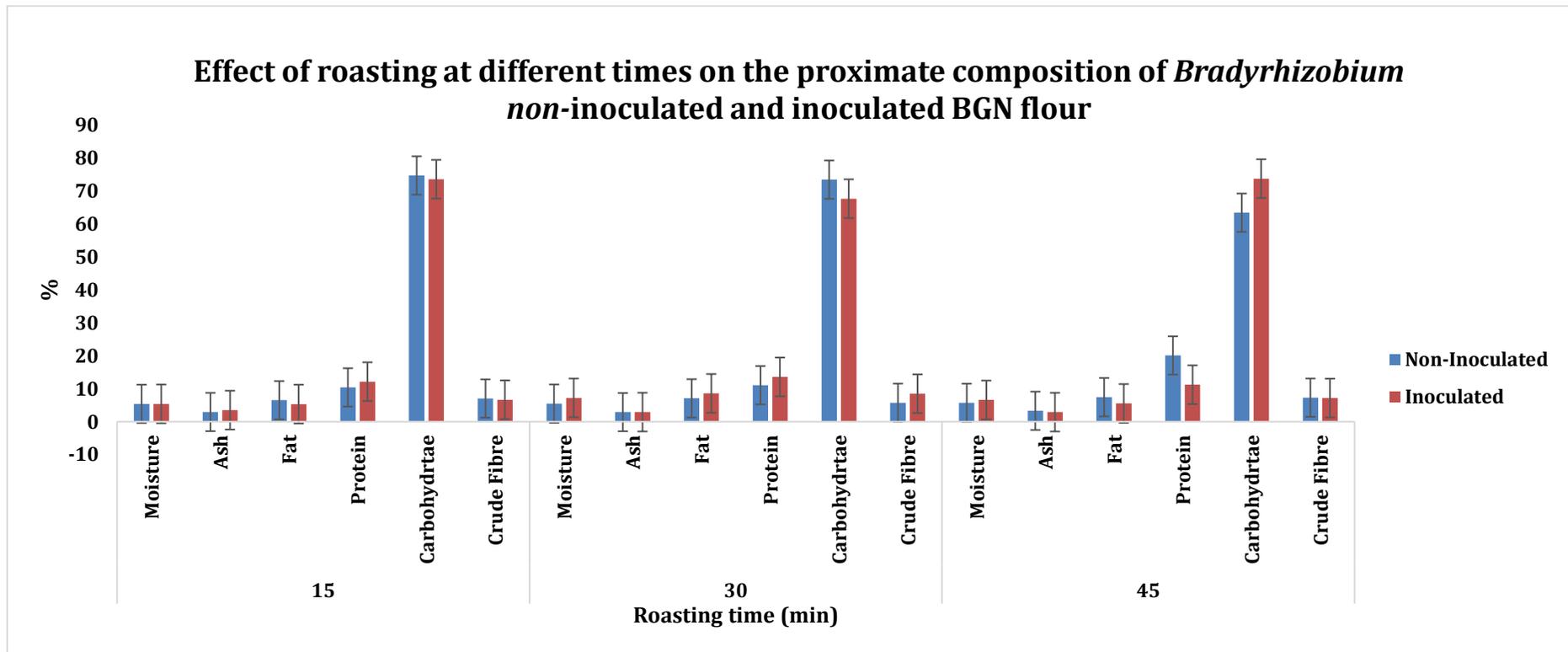


Figure 9 Effect of roasting at different times on the proximate composition of *Bradyrhizobium* non-inoculated and inoculated BGN flour

Moisture content of non-inoculated and inoculated Bambara groundnut increased with an increase in roasting time. Results in this study do not correspond to the results reported by Kavitha, & Parimalavalli (2014). Ash content in non-inoculated Bambara groundnut increased with an increase in roasting time however for inoculated Bambara groundnut there was a decrease in ash content with every increase in roasting time. The increase in ash content of non-inoculated Bambara groundnut with an increase in roasting time is in agreement with results by Byarugaba, Nabubuya, Muyonga & Mwakha (2023). Fat in non-inoculated Bambara groundnut increased with an increase in roasting time however the same trend was not observed for inoculated Bambara groundnut. The increase in fat content of non-inoculated Bambara groundnut with an increase in roasting time is in agreement with results by Byarugaba, Nabubuya, Muyonga & Mwakha (2023). Furthermore, research by Szpunar-Krok et al (2021) supports that inoculating soybean with *Bradyrhizobium* increases the polyunsaturated fatty acid which in turn increases the fat content. However, there is currently no research to support how roasting time affects the fat content. Protein in non-inoculated Bambara groundnut increased with an increase in roasting time however the same trend was not observed for inoculated Bambara groundnut. The increase in protein content of non-inoculated Bambara groundnut with an increase in roasting time is not in agreement with results by Byarugaba, Nabubuya, Muyonga & Mwakha (2023). This suggests that the protein molecules of non-inoculated Bambara groundnut are more stable to prolonged heating time of 45 minutes compared to inoculated Bambara groundnut. Carbohydrates in non-inoculated Bambara groundnut decreased with an increase in roasting time; however, the same trend was not observed for inoculated Bambara groundnut. The increase in protein content of non-inoculated Bambara groundnut with an increase in roasting time is in agreement with results by Byarugaba,

Nabubuya, Muyonga & Mwakha (2023). Crude fibre was highest (8.49%) in inoculated Bambara groundnut roasted for 30 minutes. The crude fibre of non-inoculated and inoculated Bambara groundnut was higher than those reported by Ndidi et al (2014). Carbohydrate content was highest in Bambara groundnut inoculated with *Brdayrhizobium* roasted for 45 minutes.

### **3.3.6.2 Mineral composition of Bambara groundnut flour**

From the two way interaction of inoculation treatment and time treatment, the effects of inoculation on the time treatment of the mineral composition are explained below here. Non inoculation on time treatment of Bambara groundnut was not significantly different in Ca ( $p = 0.389$ ), P ( $p = 0.128$ ), Mg ( $p = 0.423$ ), K ( $p = 0.295$ ), Na ( $p = 0.053$ ), Cu ( $p = 0.454$ ), Fe ( $p = 0.235$ ), Mn ( $p = 0.675$ ) and Zn ( $p = 0.826$ ). Inoculation on time treatment of Bambara groundnut was not significantly different Ca ( $p = 0.749$ ), P ( $p = 0.342$ ), Mg ( $p = 0.614$ ), K ( $p = 0.644$ ), Cu ( $p = 0.284$ ), Fe ( $p = 0.548$ ), Mn ( $p = 0.958$ ) and Zn ( $p = 0.340$ ) while a significant difference was observed in Na ( $p = 0.019$ ). The above results basically mean that there is a relationship between inoculation and treatment and as a result the inoculation does have an effect on time treatment.

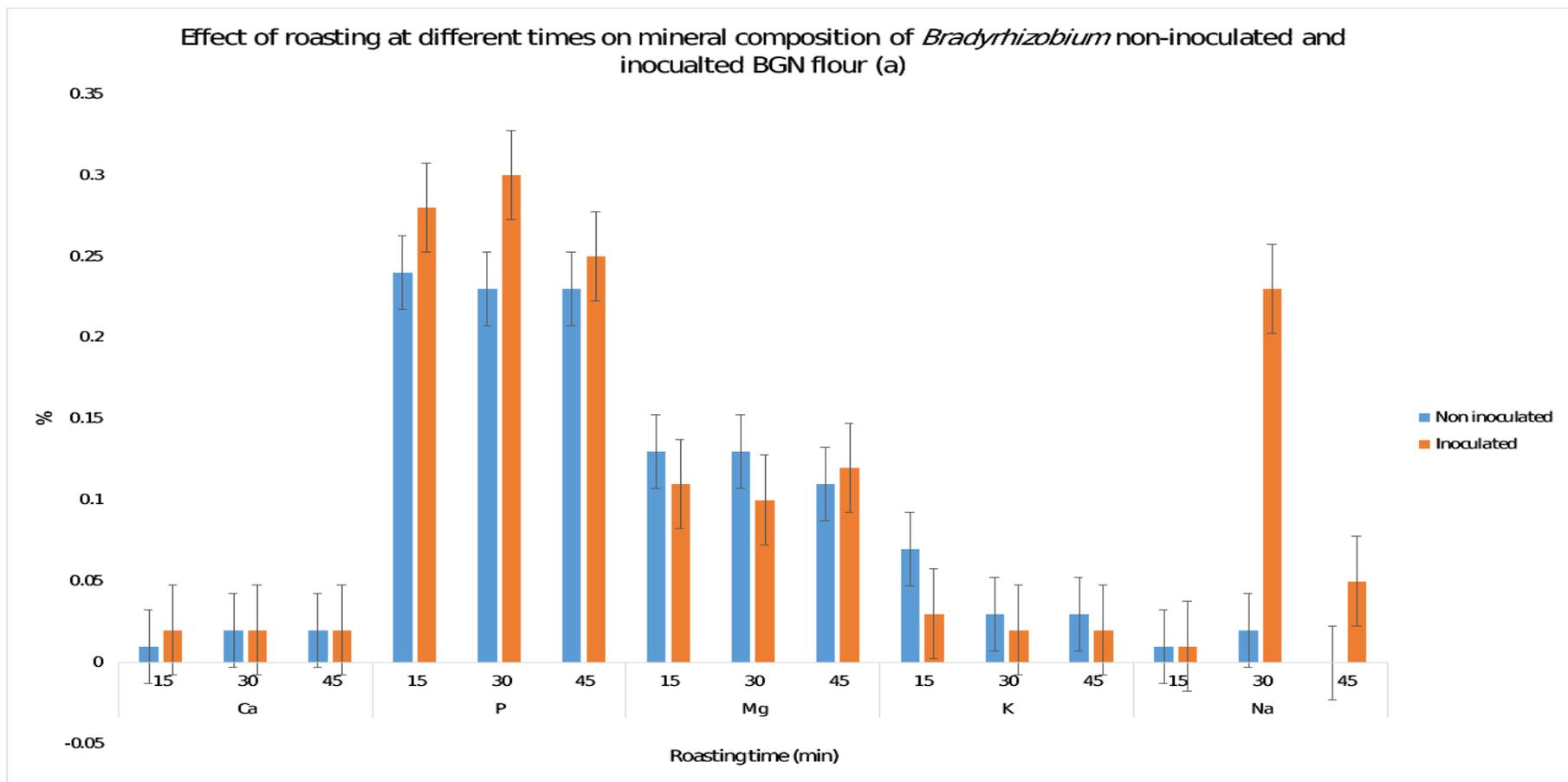


Figure 10 Effect of roasting at different times on mineral composition of *Bradyrhizobium* non-inoculated and inoculated BGN flour (a)

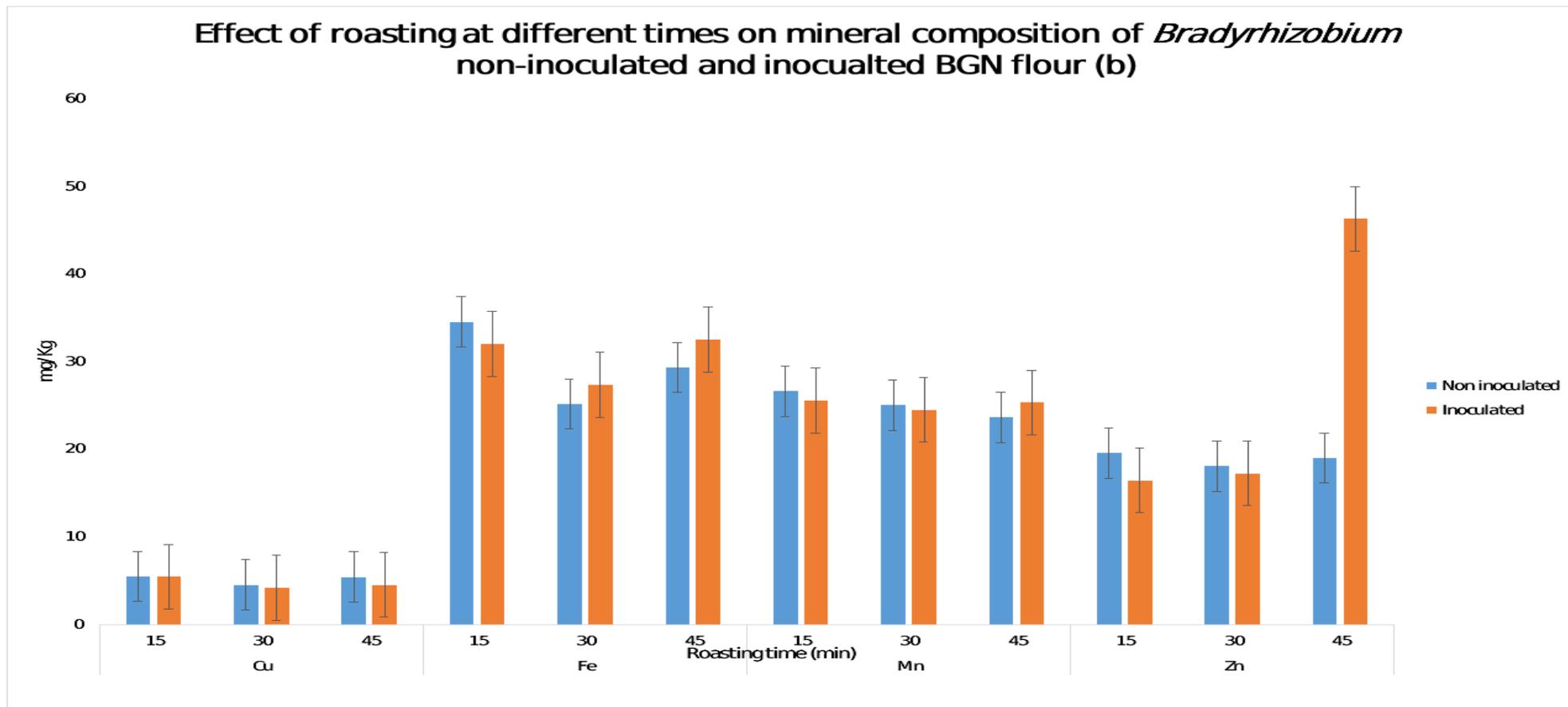


Figure 11 Effect of roasting at different times on mineral composition of *Bradyrhizobium* non-inoculated and inoculated BGN flour (b)

Calcium amongst non-inoculated and inoculated Bambara groundnut remained the same as roasting time increased. According to Lin Tan et al (202), anti-nutritional factors (ANFs) such as oxalates and phytic acid negatively affect the bioavailability of calcium in Bambara groundnut. Therefore, there is a need to study other processing methods that will effectively destroy calcium inhibiting ANFs. Phosphorus amongst non-inoculated and inoculated Bambara groundnut decreased as roasting time increased. It was also observed that phosphorus was highest in inoculated Bambara groundnut. These results agree with previous studies that inoculating Bambara groundnut with *Bradyrhizobium* increases phosphorus, magnesium and sodium (Nyoki, & Ndakidemi, 2014). Magnesium in non-inoculated Bambara groundnut decreased as roasting time increased; however, magnesium was observed to be high (0.12%) in inoculated Bambara groundnut that were roasted for 45 minutes. The magnesium values of non-inoculated Bambara groundnut reported in this study were lower than those reported by Amarteifio, Tibe, & Njogu (2006). Differences might be attributed to soil composition, plant genetics, and environmental conditions. Potassium amongst non-inoculated and inoculated Bambara groundnut cultivars decreased as roasting time increased. Potassium levels of non-inoculated BGN reported in this study were lower than those reported by Amarteifio, Tibe, & Njogu (2006). Sodium was higher in inoculated Bambara groundnut roasted for 30 minutes. Sodium levels of non-inoculated Bambara groundnut reported in this study were lower than those reported by Amarteifio, Tibe, & Njogu (2006). Copper was highest amongst non-inoculated and inoculated Bambara groundnut roasted for 15 minutes. This suggests that a roasting time of 15 minutes is sufficient in destroying phytate which is a common ANFs in Bambara groundnut that reduces copper bioavailability (Lin Tan et al., 2020). The copper levels of non-inoculated Bambara groundnut reported in this study are lower than those

reported by Amarteifio, Tibe, & Njogu (2006). Iron in non-inoculated Bambara groundnut decreased as roasting time increased, however the same trend was not observed in inoculated Bambara groundnut. Iron in inoculated Bambara groundnut roasted for 30 and 45 minutes were higher than non-inoculated Bambara groundnut roasted for 30 and 45 minutes. These results suggest that iron in inoculated Bambara groundnut is more stable and resistant to longer heating times compared to non-inoculated Bambara groundnut, however there is still a need to further research this phenomenon. The iron levels of non-inoculated Bambara groundnut reported in this study were similar to those reported by Amarteifio, Tibe, & Njogu (2006). Brea, Day, & Smith (2013) support that legumes inoculated with *Bradyrhizobium* have a high demand for iron which might subsequently increase overall iron levels in the legume. Manganese in non-inoculated Bambara groundnut decreased as roasting time increased; however, the same trend was non observed in inoculated Bambara groundnut. There are limited results on the manganese in Bambara groundnut. Zinc in inoculated Bambara groundnut increased as roasting time increased; however, the same trend was non observed in non-inoculated Bambara groundnut. The zinc levels of non-inoculated Bambara groundnut reported in this study were similar to those reported by Amarteifio, Tibe, & Njogu (2006). This suggests that longer roasting time increases the bioavailability of minerals such as zinc which softens the protein bound minerals (Hemalatha, Platel, & Srinivasan, 2007).

### **3.3.6.3 Emulsifying Activity of Bambara groundnut flour**

From the two way interaction of inoculation treatment and time treatment, the effects of inoculation on the time treatment of the emulsifying activity are explained below here. Non inoculation ( $p = 0.380$ ) and inoculation ( $p = 0.642$ ) effects on time treatment on Bambara

groundnut were not significantly different on the emulsifying activity. The above results basically mean that there is no relationship between inoculation on the time treatment and as a result inoculation does not have an effect on the salt treatment.

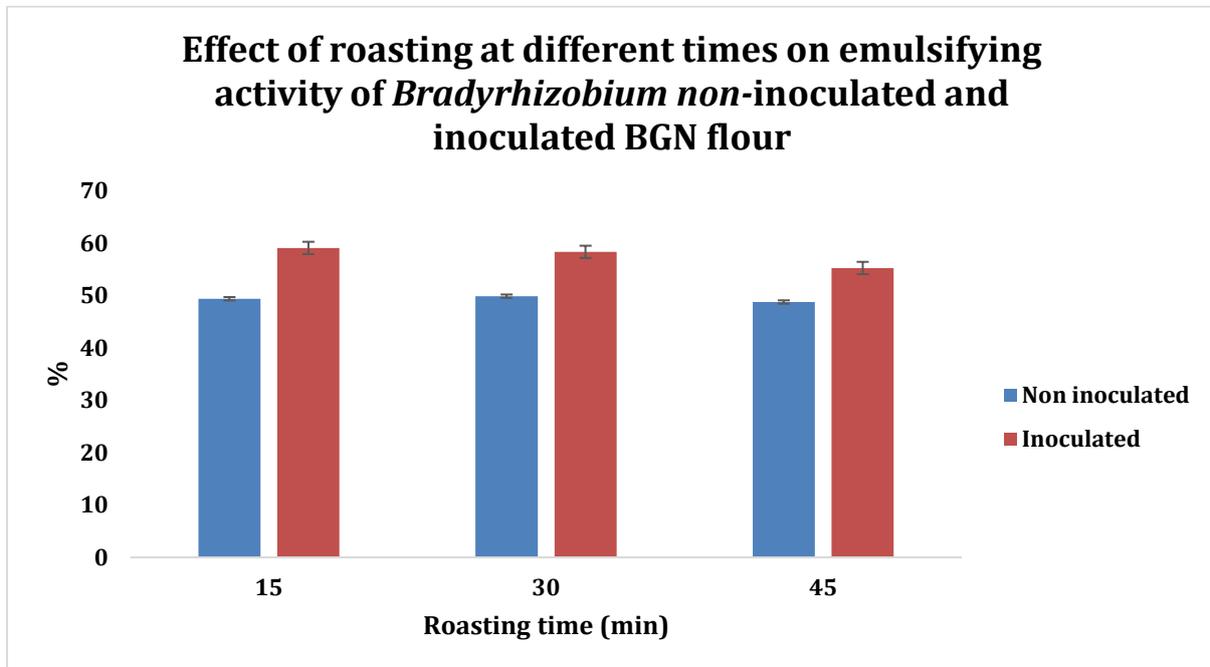


Figure 12 Effect of roasting at different times on mineral composition of *Bradyrhizobium* non-inoculated and inoculated Bambara groundnut flour

Results indicate that an increase in roasting time decreases emulsifying activity in inoculated and non-inoculated Bambara groundnut. Denaturants such as temperature and heating time can enhance the emulsifying activity of proteins in legumes (Poon, Clarke, & Schultz, 2001). This suggests that a roasting time of 15 minutes is sufficient to denature proteins present in red Bambara groundnut to an acceptable level that increases emulsifying activity.

### 3.3.7 Conclusion

In conclusion, an increase in roasting time increased fat and protein content in Bambara groundnut inoculated with *Bradyrhizobium*. However, an increase in roasting time decreased

the emulsifying property of Bambara groundnut. Different roasting times had different effects on various minerals of Bambara groundnut. It is evident that roasting methods of *Bradyrhizobium* inoculated Bambara groundnut has a potential to improve the overall nutrition. However, there is still a need to research on how different roasting times affect organoleptic features and consumer acceptability of Bambara groundnut.

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### **3.4 Effect of boiling in different salts on the proximate, mineral and emulsifying activity of *Bradyrhizobium* inoculated Bambara groundnut flour**

#### **Abstract**

Bambara groundnut (*Vigna subterranea*) is commonly referred to as a comprehensive source of nourishment, containing carbohydrate, protein, and essential amino acids. Employing the technique of boiling, which has been established as an effective approach for addressing the HTC phenomenon, the current study involved subjecting Bambara groundnuts to boiling in sodium bicarbonate, magnesium sulphate, and sodium chloride for a duration of 30 minutes. Subsequently, Bambara groundnut was subjected to drying and grinding, yielding flour. The resulting Bambara groundnut flours were then subjected to analysis encompassing proximate analysis, mineral composition and emulsifying activity. The findings of the study indicate that there exists a statistically significant disparity ( $P < 0.05$ ) between Bambara groundnut that have been boiled in different salts. Furthermore, it is evident that the method of boiling Bambara groundnut in distinct salts yields disparate effects on each nutritional composition. Significantly, it is worth noting that the addition of salts leads to a substantial increase in mineral content, with sodium chloride displaying the highest degree of emulsifying activity.

Keywords: Bambara groundnut (BGN); Cooking aids; Emulsifying activity, Hard-to-cook phenomenon; Inoculation

### 3.4.1 Introduction

*Vigna subterranea*, also known as Bambara groundnut, is a valuable source of crude protein, carbohydrates, fats, and minerals such as potassium, phosphorus, magnesium, zinc, and iron. The protein derived from Bambara groundnut has the potential to serve as an emulsifying component in the beverage industry (Alabi, Ali, Nwachukwu, Aluko, & Amonsou, 2020), offering an affordable alternative to meat and fish for vulnerable communities in Namibia (Ajilogba, Olanrewaju, & Babalola, 2022).

Bambara groundnuts exhibit a variety of types including cream, red, black, purple, and speckled seeds (Maphosa, Jideani, & Maphosa, 2022) and are cultivated by communal farmers in the northern regions of Namibia (Fleissner, 1998). Extensive research endeavours have been devoted to enhancing the productivity of Bambara groundnuts in Northern Namibia (Pasipanodya, Horn, Achigan-Dako, Musango, & Sibiya, 2022). In Namibia, conventional methods of processing Bambara groundnuts involve boiling or sun drying post-harvest. Nevertheless, other African areas have witnessed the introduction of value-added techniques for Bambara groundnuts, such as cakes, porridge, soup, coffee substitutes, and even the preservation of the seeds (Mubaiwa et al., 2016). The exposure of Bambara groundnut to boiling has been documented to be more efficient in eliminating anti-nutritional factors (ANFs) and alleviating the hard-to-cook (HTC) phenomenon (Adeleke et al., 2017).

The diminished bio-accessibility of calcium and magnesium, as well as the impaired digestibility of starch and protein, are the consequences of HTC in Bambara groundnuts (Lin Tan et al., 2020; Mubaiwa et al., 2017). While this may not present a significant challenge in terms of processing for communities that have access to advanced technological facilities, it

poses a considerable challenge for rural communities in Namibia that heavily rely on traditional methods.

The utilization of alkaline salts serves to decrease the cooking duration of legumes through the facilitation of the deterioration of pectin compounds (Tara, Christopher, & Peter, 2016). Sodium chloride, sodium bicarbonate, and *Gowa* are among the salts commonly employed for the preliminary treatment of legumes (Mubaiwa et al., 2019). It has been reported that immersing Bambara groundnuts in sodium chloride and sodium bicarbonate effectively diminishes antinutrients while concurrently increasing the mineral content, particularly calcium, magnesium, and iron (Yahaya et al., 2022). Wang et al (2020) supported that the chemical composition of magnesium sulphate has the potential to activate enzymes and induce protein precipitation, thus potentially proving advantageous for application in Bambara groundnut. This study aimed to determine the effects of *Bradyrhizobium* inoculation and salt treatment on the nutritional composition and emulsifying properties of Bambara groundnut flour grown in Namibia.

#### **3.4.2 Source of material and equipment**

Bambara groundnuts not inoculated with *Bradyrhizobium* bacteria were purchased from Namibia Institute of Seeds (NIOS) in Windhoek, Namibia. *Bradyrhizobium* bacteria inoculated with Bambara groundnut were harvested from a plantation from the University of Namibia.

### **3.4.3 Methods**

#### **3.4.1 Preparation of Bambara groundnut flour samples**

Bambara groundnuts (brown, cream and red) were boiled for 30 minutes, separately in a 0.5g/100mL solution of sodium bicarbonate, sodium chloride, and magnesium sulphate. They were then drained, dried in an oven at 60°C for 12 hours and blended into flour using a 1.8 L Hamilton Beach Food Blender.

#### **3.4.4 Analysis**

##### **3.4.4.1 Proximate analysis of Bambara groundnut flour**

Proximate content (moisture, ash fat, crude protein, carbohydrates and crude fibre) of the Bambara groundnut flour were analysed following procedures of the Association of Official Analytical Chemists (AOAC International, 1995).

##### **3.4.4.2 Mineral Analysis of Bambara groundnut flour**

The mineral composition of Bambara groundnut flour was analysed by the AAS (Atomic Absorption Spectrophotometer) except for phosphorus that was analysed on a UVS (Ultraviolet Spectrometry). Elements were prepared using the dry ashing method, then acid digested using Hydro-chloric and Nitric Acids (AOAC International, 1990; Price, 1972).

##### **3.4.4.3 Emulsification activity of Bambara groundnut flour**

Emulsification activity (EA%) of Bambara groundnut flour was determined following the adjusted methods by Mubaiwa et al. (2018). Flour samples of 0.4 g, 20 mL sunflower oil (0.9 mL/g) and 20 mL of water (1.0 mL/g) which resulted in a 1% (w/v) mixture, were prepared.

The samples were mixed for 1 minute using a vortex at 3000 rpm and centrifuged for 10 minutes at 1600 rpm. The height of the total content (TC) and the height of the emulsion layer (ELH) were measured. The EA was expressed using the equation below:

$$EA\% = \frac{\text{Height of emulsion layer}}{\text{Height of Total Content}}$$

### **3.4.5 Data Analysis**

Results were recorded as mean  $\pm$  standard deviation of duplicate measurements. Data Analysis was done using IBM SPSS Statistics (version 29, 2022, IBM Inc., USA). Multivariate analysis of variance (MANOVA) was used to establish differences between treatments. Duncan's multiple range test was used to separate means where differences exist at 5% significance level.

### **3.4.6 Results and Discussions**

#### **3.4.6.1 Proximate composition of Bambara groundnut flour**

From the two way interaction of inoculation treatment and salt treatment, the effects of inoculation on the salt treatment of the proximate composition are explained below here. Non inoculation on salt treatment of Bambara groundnut was not significantly different on moisture ( $p = 0.564$ ), ash ( $p = 0.996$ ), protein ( $p = 0.882$ ), carbohydrate ( $p = 0.957$ ) and crude fibre ( $p = 0.064$ ) while it was significantly different in fat ( $p = 0.041$ ). Inoculation on salt treatment of Bambara groundnut was not significantly different in ash ( $p = 0.091$ ), fat ( $p = 0.894$ ) and protein ( $p = 0.080$ ) while a significant effect ( $P > 0.05$ ) was observed in moisture ( $p = 0.001$ ), carbohydrate ( $p = 0.001$ ) and crude fibre. ( $p = 0.002$ ) The above results basically mean that

there is a relationship between inoculation and treatment and as a result the inoculation does have an effect on salt treatment.

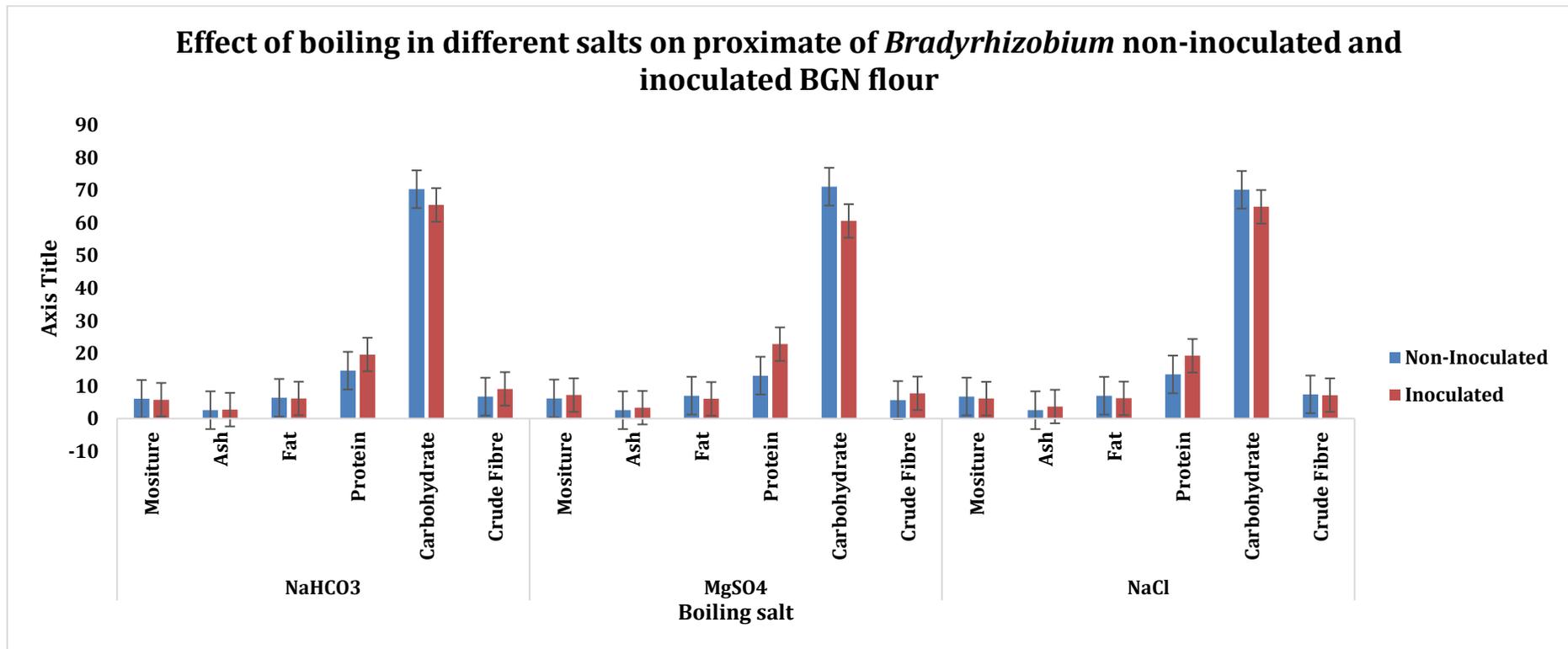


Figure 13 Effect of boiling in different salts on proximate of *Bradyrhizobium* non-inoculated and inoculated Bambara groundnut flour

Inoculated and non-inoculated Bambara groundnut boiled in sodium bicarbonate and sodium chloride had the highest carbohydrate content. The higher carbohydrate content of Bambara groundnut boiled in sodium bicarbonate may be attributed to the breakdown of carbohydrates in the seeds by amylases, whose activity is speculated to have been enhanced by the alkaline treatment, as suggested by Ibraheem et al (2023). Furthermore, sodium bicarbonate significantly diminishes the pectin, resulting in an accelerated softening of the beans (Liu et al., 2022). The disruption of the pectin is hypothesised to trigger the liberation of carbohydrate compounds, leading to an elevated carbohydrate content. Inoculated Bambara groundnut boiled in sodium bicarbonate had the highest crude fibre content (9.10%) An investigation revealed that immersing soybean, lupin, and bean seeds in a 0.5% sodium bicarbonate solution led to an increase in fibre content (El-Adawy, Rahma, El-Bedawy, & Sobihah, 2000). This finding aligns with the outcomes obtained in this study. This reduction consequently triggers an increase in crude fibre. The moisture level of inoculated Bambara groundnut boiled in magnesium sulphate was discovered to be higher (7.20%) in comparison to other salts. The protein content of inoculated Bambara groundnut boiled in magnesium sulphate was found to be higher (22.82%) compared to other salts. Notably, the application of magnesium has been shown to enhance the protein concentrations in Chinese cabbage, grain yield and crude protein concentrations in lentil, protein and oil contents in sunflower, as well as crude protein content in wheat (Ishfaq et al., 2022; Sharavdorj, Byambadorj, Jang, & Cho, 2022; Shao et al., 2021). Non-inoculated Bambara groundnut boiled in sodium chloride and magnesium sulphate exhibited an elevated (7.00 - 7.01%) fat content. Fat, an indispensable macronutrient, serves as a vital energy source, facilitates the absorption of fat-soluble vitamins, and assumes a crucial role in cellular growth and development (Murevanhema & Jideani, 2013).

### **3.4.6.2 Mineral composition of Bambara groundnut flour**

From the two way interaction of inoculation treatment and salt treatment, the effects of inoculation on the salt treatment of the mineral composition are explained below here. Non inoculation on salt treatment of Bambara groundnut was not significantly different in Ca ( $p = 0.423$ ), P ( $p = 0.124$ ), K ( $p = 0.126$ ), Na ( $p = 0.079$ ), Cu ( $p = 0.471$ ), Fe ( $p = 0.625$ ), Mn ( $p = 0.918$ ) and Zn ( $p = 0.620$ ) while a significant difference was observed in Mg ( $p = 0.0001$ ). Inoculation on salt treatment of Bambara groundnut was not significantly different Ca ( $p = 0.491$ ), P ( $p = 0.274$ ), Mg ( $p = 0.228$ ), K ( $p = 0.948$ ), Na ( $p = 0.104$ ), Cu ( $p = 0.616$ ) and Mn ( $p = 0.165$ ) while a significant difference was observed in Fe ( $p = 0.020$ ) and Zn ( $p = 0.039$ ). The above results basically mean that there is a relationship between inoculation and treatment and as a result the inoculation does have an effect on salt treatment.

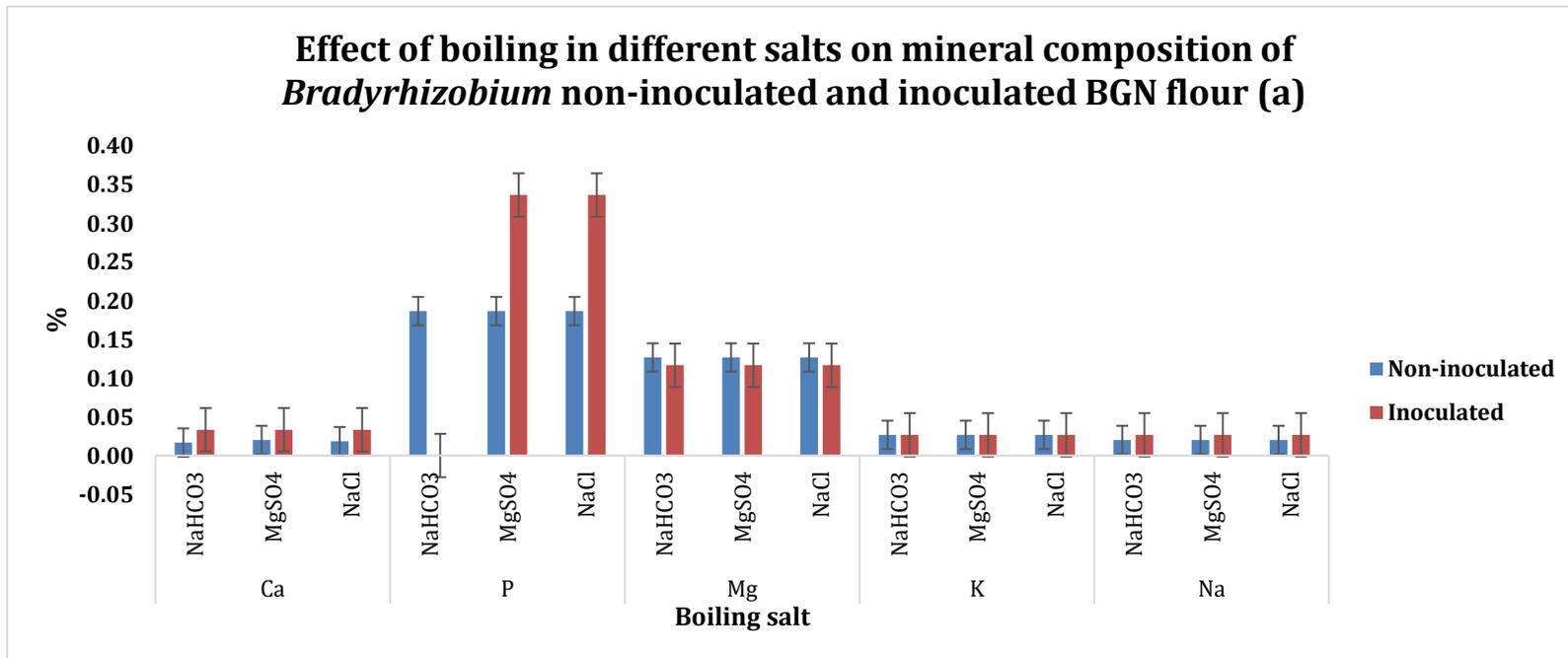


Figure 14 Effect of boiling in different salts on mineral composition of *Bradyrhizobium* non-inoculated and inoculated *Bambara groundnut* flour (a)

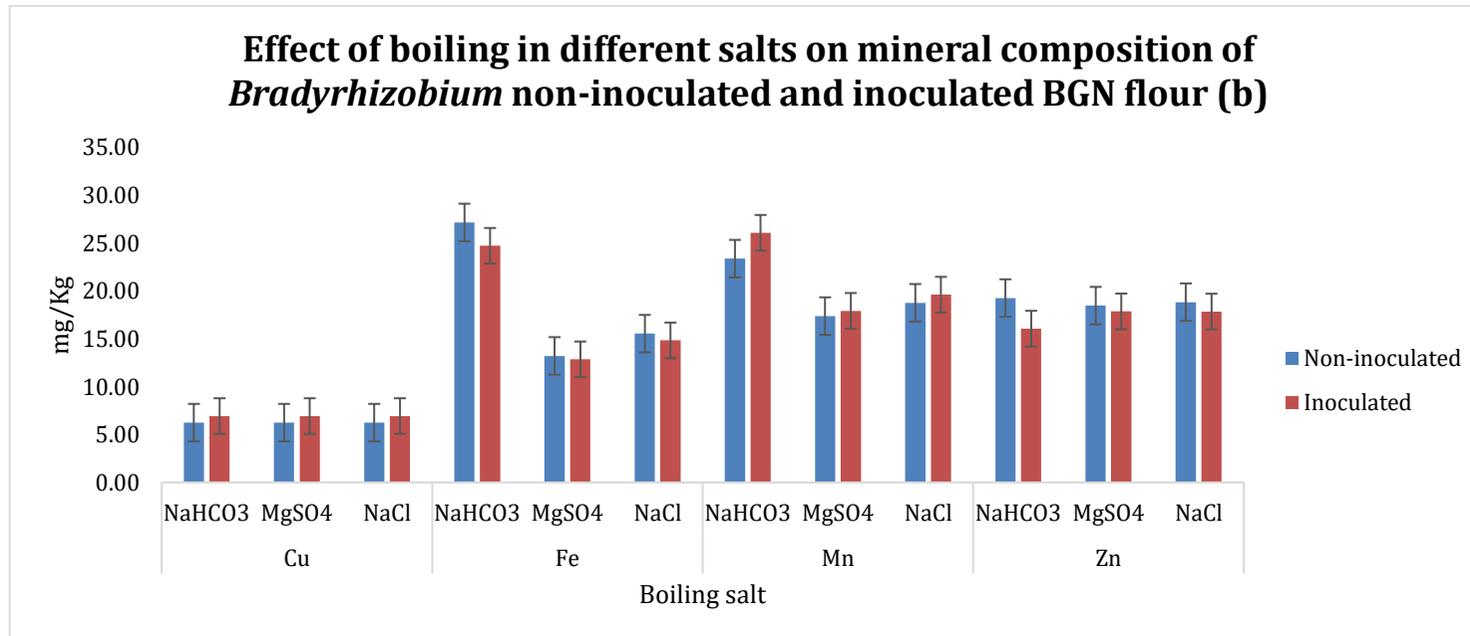


Figure 15 Effect of boiling in different salts on mineral composition of *Bradyrhizobium* non-inoculated and inoculated Bambara groundnut flour

(b)

Inoculated Bambara groundnut boiled in sodium bicarbonate had the highest sodium. This suggests that during boiling, sodium ions in the boiling solution are transferred to the components of the Bambara groundnut thereby elevating sodium levels. To our knowledge there currently exists limited studies on the effects of boiling Bambara groundnut or any other legume in magnesium sulphate. Inoculated Bambara groundnut boiled in magnesium sulphate had the highest phosphorus, potassium and iron. Inoculated brown Bambara groundnut boiled in table salt was found to contain elevated levels of phosphorus. Mandizo & Ondido (2019) discovered that Bambara groundnut had a higher concentration of phosphorus, potassium and magnesium in the cotyledon of the legume. This explains that table salt was effective enough to penetrate the seed coat and release trapped phosphorus. Additionally boiling in sodium bicarbonate was found to increase pH of the solution and decrease polyphenols (Faye et al., 2023). A study by Shaban et al (2019) found that white kidney beans fertilised with magnesium sulphate displayed a higher level of magnesium and zinc. Table salt solution contains Na<sup>+</sup> and Cl<sup>-</sup> ions that can cause the migration of ions (Widodo, Sela, & Santosa, 2018) through the process of diffusion.

#### **3.4.6.3 Emulsifying Activity of Bambara groundnut flour**

From the two way interaction of inoculation treatment and salt treatment, the effects of inoculation on the salt treatment of the emulsifying activity are explained below here. Non inoculation ( $p = 0.253$ ) and inoculation ( $p = 0.979$ ) effects on salt treatment on Bambara groundnut were not significantly different on the emulsifying activity. The above results basically mean that there is no relationship between inoculation on the salt treatment and as a result inoculation does not have an effect on the salt treatment.

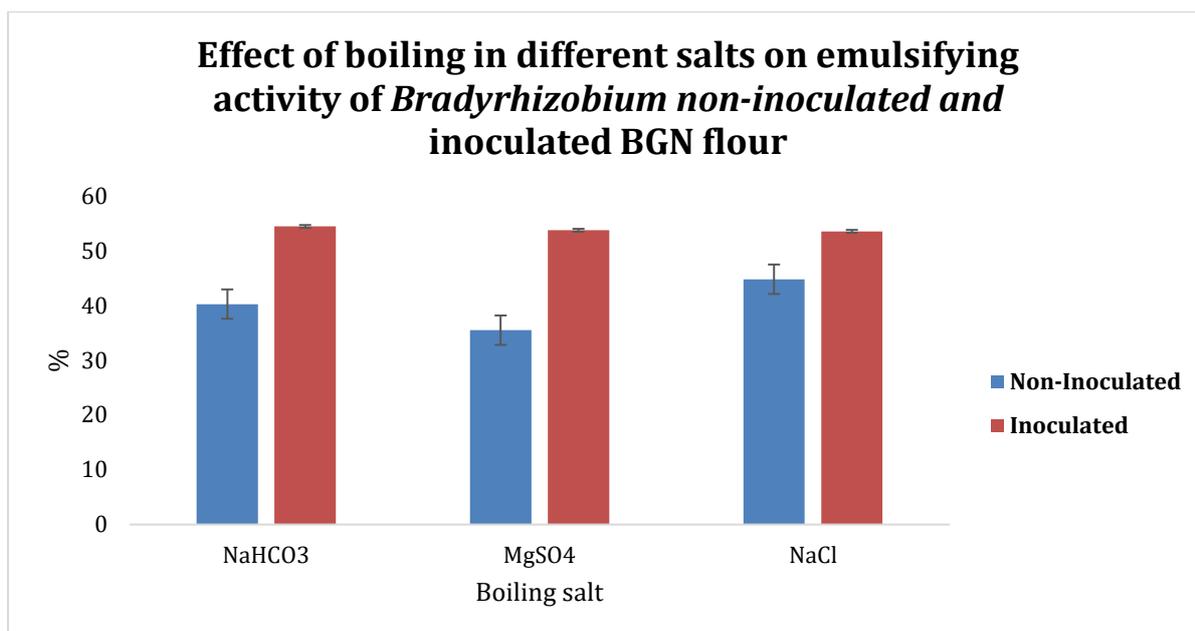


Figure 16 Effect of boiling in different salts on emulsifying activity of *Bradyrhizobium* non-inoculated and inoculated Bambara groundnut flour

The emulsifying activity (EA) was observed to be highest in all inoculated Bambara groundnut cultivars boiled amongst all three salts (62.48 - 64.71%). However inoculated Bambara groundnut boiled in sodium chloride displayed the highest emulsifying activity (64.71%). The high emulsifying activity in inoculated Bambara groundnut is due to the high protein and crude fibre content in inoculated Bambara groundnut. According to Benjamin, Silcock, Beauchamp, Buettner, & Everett, (2014) legumes with a high protein and crude fibre exhibit good emulsifying activities. EA is affected by pH and ionic strength of the boiling salt (Ettoumi, Chibane, & Romero, 2016).

### 3.4.7 Conclusion

It is evident that boiling Bambara groundnut with different salts differently affects its nutritional composition. Sodium chloride is the most effective alkaline salt in improving the

EA of boiled Bambara groundnut. In terms of processing, using such sodium chloride salt would be cost effective and easily accessible to vulnerable communities as compared to sodium bicarbonate and magnesium sulphate. There is a need to further study the effects of boiling and other alkaline treatment on other sugars within the Bambara groundnut. This information will be important to indicate if Bambara groundnut will be a suitable recommendation for diabetic patients. It is evident that inoculation greatly affects the nutrition of Bambara groundnut. Inoculated beans had a greater nutritional profile compared to non-inoculated beans. Information on proteins isolated from Bambara groundnut may provide more insight on the different functional properties. Knowledge on the functional properties (water absorption capacity, oil absorption capacity, swelling capacity, foam capacity and stability, emulsifying capacity and stability, and protein solubility) as highlighted by Adewumi, Felix-Minnaar, & Jideani (2022) will advise product developers on which food product to manufacture.

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## **Chapter 4: General Discussion**

This chapter discusses the observed relationship between Bambara groundnut cultivars and different roasting times in terms of sensory attributes.

### **4.1 Relationship between roasting and sensory attributes of Bambara groundnut flour**

During roasting of the Bambara groundnut cultivars, the following changes were observed amongst the Bambara groundnut that were roasted:

#### **4.1.2 Roasting time effects on the colour of Bambara groundnut flour**

During the experiment it was observed that a longer roasting time resulted in a darker colour of the Bambara groundnut flour as highlighted in figures 17, 18 and 19 below. Bambara groundnut that was roasted for 45 minutes had a darker colour than Bambara groundnut roasted for 15 and 30 minutes. This suggests that roasting times cause colour changes in Bambara groundnut. In a study by (Mridula, Goyal, Bhargav, & Manikantan, 2007), it was found that roasting soybeans affected the colour which explained that legumes that were roasted for longer periods and higher temperatures were darker. However, it must be noted that legumes roasted for longer periods may result in poor sensory acceptability. The change in colour of Bambara groundnut, as a result of the process of roasting, can be attributed to non-enzymatic browning, which is closely linked to the occurrence of the Maillard reaction (Koriyama, Teranaka, Tsuchida, & Kasai, 2022). The Maillard reaction is a series of chemical reactions that occur when amino acids and reducing sugars are heated together, resulting in the browning of legume and the creation of new flavours and aromas (Adetokunboh, Obilana, & Jideani, 2022).



Figure 17 Bambara groundnut roasted for 15 minutes



Figure 18 Bambara groundnut roasted for 30 minutes



Figure 19 Bambara groundnut roasted for 45 minutes

#### **4.1.2 Aromatic changes in Bambara groundnut due to roasting**

During the experiment it was observed that a longer roasting time resulted in a nutty and pungent smell of the Bambara groundnut flour. Bambara groundnut that was roasted for 45

minutes had a more nutty and pungent smell than Bambara groundnut roasted for 15 and 30 minutes. Roasting legumes can affect their smell due to the formation of aroma compounds during the process (Schenker et al., 2002; Baggenstoss, Poisson, Kaegi, Perren, & Escher, 2008). Longer roasting times lead to deeper flavours and aromas (Adetokunboh, Obilana, & Jideani, 2022). The nutty flavour in Bambara groundnut roasted for 45 minutes comes from the Maillard reaction (Adetokunboh, Obilana, & Jideani, 2022). Pyrazines, polyphenolic compounds, fatty acids methyl esters, and hydrocarbons all contribute to the aroma of Bambara groundnut and respond differently to roasting (Adetokunboh, Obilana, & Jideani, 2022; Okafor, Jideani, Meyes, & Rose-Hill, 2022). High roasting intensities result in more pyridines and pyrroles, which contribute to different aromas in legumes including Bambara groundnut (Moon, & Shibamoto, 2009).

#### **4.1.3 Relationship between roasting and colour of Bambara groundnut**

During the experiment it was observed that darker coloured Bambara groundnut resulted in darker coloured flour after roasting (as presented in figure 20, 21 and 22). Red Bambara groundnut roasted for 30 and 45 minutes had a darker colour of the flour compared to cream and brown Bambara groundnut. Different bean varieties may have different levels of aromatic compounds, which can impact the final flavour and aroma of the roasted beans (Mishra, Tripathi, Gupta, & Variyar, 2019). A study discovered that roasting darker coloured soybeans and lentils can enhance the brown colour due to Maillard reaction (Kim et al., 2011; Mirali, Purves, & Vandenberg, 2017). This suggests that the seed coat of Bambara groundnut affects the colour of flour.



Figure 20 Bambara groundnut cultivars roasted for 15 minutes



Figure 21 Bambara groundnut cultivars roasted for 30 minutes



Figure 22 Bambara groundnut cultivars roasted for 45 minutes

## 4.2 References

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## **Chapter 5: General Conclusion and Recommendations**

Namibia is experiencing severe food insecurity problems. Results in this study proved that inoculated Bambara groundnut could enhance food security by improving nutrient content, due to the rich protein, fibre and essential minerals.

- Different Bambara groundnut cultivars displayed different nutritional profiles in response to boiling, roasting and *Rhizobial* inoculation. Findings in this research suggest that inoculated Bambara groundnut can serve as a healthy food for vegan and diabetic people due to its excellent protein and carbohydrate content.

It is important to note that inoculated Bambara groundnut had the highest emulsifying properties. This suggests that it can be used as an emulsifier in vegan sausages. Furthermore, there was a reduction in tannins in the processed cream Bambara groundnut. Condense tannins could not be detected in brown and red Bambara groundnut.

### **5.1 Recommendations for Bambara groundnut in response to its future potential uses**

- Training and research

Future research should prioritise improving the productivity, resilience, and economic value of Bambara groundnut through the development of innovative technologies, practices, and markets. Effective conferences, workshops, and training programs should be organised to promote capacity building and facilitate knowledge exchange among researchers, farmers, and other stakeholders involved in Bambara groundnut production and utilisation (Khan, Rafii, Ramlee, Jusoh, & Al-Mamun, 2021). Future research should study the possible effects in the use of a combination of different salts used including boiling and roasting. Instead of using colour to identify Bambara groundnut, it is advised to identify the names of the Bambara groundnuts cultivars and landraces that will be considered for use for processing. In Namibia

there is a need to inform consumers of the difference between Bambara groundnut and other legumes commonly grown in Namibia. Furthermore, training should focus on creating awareness amongst farmers on correct storage and post-harvest methods to reduce the HTC phenomenon that is prevalent in Namibia's hot and dry conditions. This study does provide evidence that different BGN cultivars differ in the nutritional profile. It is believed that the seed coat and colour have a role to play in these differences. Therefore, further research is needed to highlight the antioxidant, vitamins and extensive ANFs profile of the different Bambara groundnut cultivars. Information on the cultivars will help food manufacturers determine which cultivar is beneficial for a particular age group to consume. In addition, there is significant potential for increasing the seed size and nutritional quality of Bambara groundnut through inoculation. This approach aims to elevate the levels of protein, unsaturated fatty acids, and essential micronutrients in the crop (Lin Tan et al., 2020; Khan, Rafii, Ramlee, Jusoh, & Al-Mamun, 2021).

- Educational, financial, government institution and policy makers

Encouraging the involvement of financial and technical support for researchers and institutions engaged in BGN research is crucial (Khan, Rafii, Ramlee, Jusoh, & Al-Mamun, 2021). Educating the nation on BGN potential should not only be left in the hands of the government, however important stakeholders such as universities, vocational training colleges and research institutions (i.e. National Commission Research Science and Technology, Namibia Scientific Society, National Botanical Research Institute and Namibia Green Hydrogen Institute) should collaborate. This support will accelerate the adoption and development of innovative technologies and practices in Bambara groundnut production and utilisation. To facilitate

collaboration and knowledge sharing among researchers and stakeholders, the establishment of a comprehensive research network and platform at the national, regional, and international levels is paramount. This network will provide a platform for researchers to exchange ideas, foster cooperation, and advance Bambara groundnut research and development.

- *Bradyrhizobium* inoculation

The inoculation of legumes with nodulating *Bradyrhizobium* holds immense promise in substantially bolstering addressing food security in Namibia. There is a need to research affordable and effective *rhizobacteria* strains that can improve the nutrition capacity of Bambara groundnut and other legumes grown in Namibia.

- Processing

Bambara groundnut exhibits promising prospects to produce value-added products, including snacks, flour, and animal feed. Future research endeavours should concentrate on developing novel technologies and exploring new markets to enhance the economic value of Bambara groundnut and its contribution to rural livelihoods. These processing methods should be low cost and easily accessible methods that can be employed at urban and semi-urban and advanced areas in Namibia. This means that different genotypes of the plant can exhibit varying levels of fat content, which can have implications for its overall composition and potential health benefits. For instance, a recent study conducted by Mustiga et al. (2019) discovered that genotypes of cocoa beans with higher fat content exhibited a distinct fatty acid profile when compared to genotypes with lower fat content. This finding suggests that the genetic makeup of a particular legume species can play a significant role in determining its fat content and subsequent nutritional properties. Furthermore, it is worth noting that the environment in which legumes are cultivated can also impact their fat content. In the case of cocoa beans, another

study conducted by Mustiga et al (2019) revealed that the fat content of these beans can vary depending on the specific climate and soil conditions in which they are grown. This suggests that factors such as temperature, rainfall, and soil composition can all influence the fat content of legumes, including Bambara groundnut. Understanding these environmental factors is crucial for farmers and researchers alike, as it allows for the development of cultivation strategies that can optimise the fat content and overall nutritional quality of legumes. By better understanding the factors that contribute to low fat content in Bambara groundnut, we can further enhance our knowledge of the nutritional composition and potential health benefits associated with legumes, and ultimately contribute to the development of sustainable and nutritious food systems. Processing techniques can also have an impact on the fat composition of legumes. For example, research where Bambara groundnut was boiled in water for 25 minutes and steamed for 20 minutes resulted in the concentration of the endosperm which increased the fat content (Elochuckwu, 2020). An illustrative study conducted by Celmeli et al (2018) discovered that the process of fermentation resulted in an increase in palmitic, stearic, and oleic acids within the fatty acid profile of bean-based plant substitute for fermented milk, when compared to the composition observed in raw beans. It is important to note that legumes are characterized by their low-fat content, as well as their absence of saturated fats and cholesterol (Polak, Phillips, & Campbell, 2015). The fat levels in Bambara groundnut can vary significantly, ranging from 1.4% to 12%, depending on the variety and the prevailing growing conditions (Lin Tan et al., 2020; Maphosa, Jideani, & Maphosa, 2022; Hlanga, Modi, & Mathew, 2021; Jideani, Jideani, Jideani, & Jideani, 2021). On average, Bambara groundnut harbours approximately 6.5% fat (Lin Tan et al., 2020; Murevanhema, & Jideani, 2013).

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## Appendices

### Appendix 1



#### ETHICAL CLEARANCE CERTIFICATE

**Ethical Clearance Reference Number:** NEC029

**Date:** 28/06/2024

This Ethical Clearance Certificate is issued by the University of Namibia Decentralized Ethics Committee (DEC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the ethics committee.

**Title of Project:** "THE EFFECT OF BOILING AND ROASTING ON THE NUTRITIONAL COMPOSITION, EMULSIFYING PROPERTY AND ANTI-NUTRITIONAL FACTORS OF INOCULATED AND NON-INOCULATED VIGNA SUBTERRANEA (BAMBARA GROUNDNUT)"

**Principal researchers:** MARIA N.P KAPEMBE

**Student number:** 201703063

**Remarks:** the research proposal respects all the parameters to obtain the ethical clearance

#### Centre for Research Services

Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the ethics committee. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the ethics committee
3. The Principal Researcher must report issues of ethical compliance to the ethics committee (through the Chairperson) at the end of the Project or as may be requested by the ethics committee
4. The ethics committee retains the right to:
  - i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
  - ii) Request for an ethical compliance report at any point during the course of the research.

The ethics committee wishes you the best in your research.

A handwritten signature in black ink, appearing to read "J. Yabe", is written over a horizontal line.

Dr. John Yabe (Chairperson Neudamm Decentralized Ethics Committee)

A handwritten signature in black ink, appearing to read "D. Mumbengegwi", is written over a horizontal line.

Prof. Davis Mumbengegwi (Head, Multidisciplinary Research)

## Appendix 2



Figure 23 Brown Bambara groundnut cultivars



Figure 24 Cream Bambara groundnut cultivars



Figure 25 Red Bambara groundnut cultivars



Figure 26 Sodium bicarbonate (bicarbonate of soda), sodium chloride (table salt), magnesium sulphate (epsom salt)