Cape Peninsula University of Technology

Functionality of gum Arabic and sodium alginate on selected properties of Bambara groundnut tofu and its chunks

by

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DECLARATION

I, **Londiwe Mary Chipeta,** declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

Bambara groundnut (BGN) (*Vigna subterranea* [L.] Verdc.) is a source of protein (15-25%), making this legume a suitable alternative to soybean for tofu production. The use of BGN as a main ingredient in tofu is not documented, as most tofu is made from soy milk extracted from soaked soybeans. The texture (Instron texture analyser), colour (HunterLab ColorFlex spectrophotometer), rheology (rheometer), proximate (AOAC methods) and microstructure (scanning electron microscope) of BGN tofu as affected by coagulants (vinegar, lemon juice and gluconolactone (GDL)) and hydrocolloids (gum Arabic and sodium alginate) were established in this study. Factorial design and response surface methodology were used to optimise gum Arabic and sodium alginate concentrations at 0.3, 0.4 and 0.5%. The interaction of gum Arabic and sodium alginate was optimal at 0.5%. The combination of gum Arabic and sodium alginate significantly ($p < 0.05$) increased the hardness (9.15%) and springiness (11.46%) of tofu and decreased its gumminess (19.72%). The lightness of BGN tofu increased as GDL concentration increased. The coefficient of determination (R^2) for all measurements using the Power law was >0.9. The consistency coefficient (K) of BGN milk extract prepared using GDL with gum Arabic and sodium alginate was >1, indicating shear thickening behaviour. The entrapped water content of BGN tofu ranged from 45.13 (1% GDL-tofu) to 65.60% (0.6% GDL-tofu + 0.5% GA + 0.5% SA). Adding gum Arabic and sodium alginate to BGN tofu increased the protein, ash and carbohydrate content and decreased the moisture content. Vinegar-tofu exhibited a uniform, smaller particle size network and smooth surface. Gum Arabic and sodium alginate had a significant ($p < 0.05$) positive effect on the functional and sensory qualities of BGN tofu chunks. Leucine (4.34-4.94%) and phenylalanine (4.61-5.62%) were the most abundant essential amino acids in all BGN tofu chunks samples. There was an increase in the protein content of BGN tofu when gum Arabic and sodium alginate ratio was incorporated. Bambara groundnut tofu chunks with 0.6% GDL with gum Arabic and sodium alginate received the highest in appearance, colour, taste, texture and overall acceptability than tofu chunks coagulated with vinegar and 0.6% GDL. The results of this study highlighted the potential of BGN tofu and its chunks as a nutritious, cholesterol-free protein alternative, with the quality and acceptability influenced by coagulant types and hydrocolloid concentrations.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

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GLOSSARY

Acronym	Definition/Explanation
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
BGN	Bambara groundnut
BGNF	Bambara groundnut flour
C.V	Coefficient of variation
DDT	Dithiothreitol
GDL	Gluconolactone
GA	Gum Arabic
MANOVA	Multivariate analysis of variance
SEM	Scanning electron microscope
SA	Sodium alginate
7S	Vicilin
11S	Glycinin
K	Consistency coefficient
WAC	Water absorption capacity
N	Flow behaviour index
R^2	Coefficient of determination
L*	Lightness
+a*	Redness
-a*	Greenness
+b*	Yellowness
-b*	Blueness

CHAPTER ONE

MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Legumes have garnered significant interest for incorporation into various food products because of their broad availability and affordability (Arise, 2016; Polak *et al.*, 2015; Bouchenak & Lamri-senhadji, 2013). Legumes are part of the Leguminosae family and are primarily grown for their edible seeds (Iqbal *et al.*, 2006). They play an essential role in the human diet due to their high content of protein, carbohydrates, minerals, and vitamins (Bouchenak & Lamri-senhadji, 2013). Mohammed *et al.* (2016) and Eine (2016) emphasised that incorporating grain legumes into cereal-based diets can enhance their protein content and overall nutritional value. Among legumes, soybean is a well-known source of plant-based protein and offer numerous health benefits.

Soybean contains therapeutic compounds such as phytonutrients (flavonoids and phytoestrogens). The antioxidants in soybeans are good for neutralising the free radicals present in the human body that cause cancer. However, soybeans are associated with endocrine disorders and soy products are listed as a major food allergen by the FDA (USA) labelling regulations (Sanjukta & Rai, 2016; Selb *et al.*, 2017; Naresh *et al*., 2019). Furthermore, soybean dust can cause health problems related to lung irritancy (Naresh *et al*., 2019). Despite these health concerns associated with soybeans, tofu remains a popular ingredient in many dishes because of its high protein and carbohydrate content (Dey *et al.*, 2017).

Tofu is made by coagulating boiled soy milk using salt and acid as coagulants. This coagulation process produces a protein gel that traps water, soy lipids and other components in the matrix, generating curds. Pressing then separates the curds from the whey, resulting in white and soft blocks of curds (Murdia & Wadhwani, 2010). The gelling process of tofu begins with the application of heat, denaturing soy protein and revealing its hydrophobic region (Huang & Kuo, 2014). The denatured proteins self-associate by means of non-polar interactions, hydrogen bonds and disulphide bonds. Protein interactions form complexes with negatively and positively charged surfaces over a wide pH range. Coagulation of hydrophobic proteins is facilitated by calcium ions present in acids and salts. The neutralisation of negatively charged soy protein aggregates is achieved by adding a coagulant.

Studies by Shen & Kuo (2017) and Murad *et al.* (2015) identified carrageenan, gum Arabic and corn starch as alternatives to improving the texture profile of tofu. Carrageenan promotes the localised coagulation of proteins, forming tofu with a coarser and firmer texture. Carrageenan can be used to improve textural properties of tofu by varying its concentration (Shen & Kuo, 2017).

The β-conglycinin (7S) and glycinin (11S) fractions are the major storage proteins found in soybeans (Syah *et al.*, 2015). Soy globulins are insoluble within their isoelectric point range (pH 4.5) and account for approximately 90% of proteins in water or other aqueous solvents (Barac & Jovanovic, 2004; Yuan *et al.,* 2002). Consequently, the composition of β-conglycinin and glycinin has been shown to influence the textural properties of tofu products (Chang & Liu, 2012; Taški-Hajdukovic *et al*., 2014). Comparably, the fraction of vicilin (7S) has also been found to be the large storage protein fraction of Bambara groundnut (BGN) (*Vigna subterranean* (L.) Verdc) (Okpuzor *et al*., 2010; Anjum *et al.*, 2011). It comprises of 71% albumin, 23% globulin, 2.2% prolamins, and 5.2% glutelins (Gulzar & Minnaar, 2017). Bambara groundnut not only shares similar storage proteins with soybean but is also relatively high in protein (15-25%) and carbohydrates (63%) (Abdua *et al.*, 2012). Among beans, the concentration of dissolved dietary fibre in BGN seeds is greater (Gonné *et al*., 2013). Dietary fibre plays a role in reducing the risk of conditions such as heart disease and gastric cancer (Mkandawire, 2007). Soy and BGN can be used in the production of meat substitutes such as textured vegetable protein (TVP).

Textured vegetable protein, or soy mince, is defatted soy flour that undergoes mechanical processing through an extruder to develop its structural integrity and distinctive chewy texture when rehydrated and cooked. These granular dried products are produced from highly refined defatted soybean meal. The choice of raw materials for TVP depends on factors such as availability as well as functional, nutritional and physiological properties (Anjum *et al.,* 2011). Soybeans are commonly used to produce textured protein (Riaz, 2011); however, this study aims to investigate the potential of BGN tofu in the production of chunks (chewy mince-meat like product).

1.2 Statement of the Research Problem

Tofu is a nutrient-rich food product that is consumed worldwide. It is traditionally produced by curdling boiled soymilk through the addition of coagulants such as salt or acid, and then pressing the curd into a tofu mould to remove the whey (Murdia & Wadhwani, 2010). A novel and preliminary study where the effect of processing parameters on the texture and yield of BGN tofu was investigated by Chipeta & Jideani (2017). This made BGN a potential alternative to soybeans for tofu production. However, upon storage, BGN tofu had high syneresis which resulted in the destruction of its structure and a decrease in its water-holding capacity which reduced its shelf-life and sensory qualities. Therefore, there was a need to improve the textural properties of BGN tofu. Hydrocolloids could be used to mitigate this undesirable occurrence. The primary function of hydrocolloids in tofu is to improve its texture and water retention, thereby extending its shelf life, as reported by Murad *et al.* (2015) and Shen & Kuo (2017). As such, although BGN tofu was previously studied (Chipeta & Jideani, 2017), nothing is known about the effect of hydrocolloids on its properties. Investigating the effect of gum Arabic (GA) and sodium alginate (SA) on the textural, rheological and physicochemical properties of BGN tofu will address this knowledge gap.

1.3 Objectives of the Research

1.3.1 Broad objective

The main objective of this research was to evaluate the functionality of gum Arabic and sodium alginate on the textural, rheological and physicochemical properties of BGN tofu and tofu chunks.

1.3.2 Specific objectives

The specific objectives of this research were to:

1. Optimise the concentration of gum Arabic and sodium alginate for optimal BGN tofu and establish its textural, rheological and physicochemical properties.
2. Produce and characterise the functional and consumer acceptability of BGN tofu chunks.

1.4 Hypotheses

The hypotheses that were tested in this research were:

1. Gum Arabic and sodium alginate will significantly affect the textural, rheological and physical properties of BGN tofu.
2. BGN tofu chunks with desirable functional and sensory qualities will be produced from the optimal BGN tofu.

1.5 Delimitations of the Study

The limitations of this research were:

1. Bambara groundnut seeds were used as received without sorting into colours.
2. Only two hydrocolloids were used, namely, gum Arabic and sodium alginate.

1.6 Significance of the Research

This project falls under the Cape Peninsula University of Technology research strategy of bioeconomy aimed at producing value-added products towards poverty alleviation, gender equality, and human capital development. The production of BGN tofu and chunks will expand the food uses of BGN by creating a demand to increase the cultivation of the legume. Furthermore, the successful commercialisation of BGN will lead to higher consumer demand hence creating job opportunities, consequently supporting livelihoods. This study will also promote gender equality and the empowerment of women since BGN is mostly grown by women. This study will promote the economic stability of BGN farmers and help them improve their quality of life. Versatile products such as BGN tofu play a crucial role in encouraging healthier lifestyles and diets as well as reducing substantial amounts of food losses. The high protein content of BGN tofu will contribute to eradicating malnutrition and transforming food systems. Since BGN is inexpensive, low-income groups can also afford it thus making nutritious diets available to all. In South Africa, BGN's major production areas are spread across Mpumalanga, Limpopo, KwaZulu-Natal, Gauteng and North West provinces. Therefore, using this legume for tofu production will not only increase its utilisation but also promote job creation, thus alleviating poverty and encouraging its cultivation in other provinces in South Africa. Additionally, new knowledge and improved skills will be generated by the use of BGN protein curd as the main ingredient in tofu chunks production. This research will also lead to the completion of a Master's degree which positively impacts the postgraduate output of the Cape Peninsula University of Technology, thereby impacting human capacity building.

1.7 Expected Outcomes, Results and Contributions of the Research

The outcomes of this research will give insight into the application of BGN in the production of tofu, providing the industry with an alternative to soybean. The addition of hydrocolloids is expected to significantly modify the textural characteristics of tofu. The effect of gum Arabic and sodium alginate on tofu hardness, gumminess and springiness will provide knowledge of hydrocolloid-protein interactions as well as the nature and behaviour of tofu when exposed to different conditions. The results of the effect of different concentrations of hydrocolloids on the syneresis of BGN tofu and on the overall physicochemical and functional properties of the resultant tofu will allow for successful optimisation. An innovative tofu-like product from BGN that is aligned with food safety and customer acceptability will be produced. New knowledge will be documented, paving the way for increased availability of plant-based protein products. A chewy mince-meat like product (chunks), with a relatively high protein content and good quality, will be produced. Finally, a unique value-added product from BGN will be produced. There is no work documented on BGN chunks production thus, new skills and processing technology will be developed. At least one article will be published in an accredited journal, and the research output will be presented in at least one national or international conference. A Master's degree is also expected from this research study.

1.8 Thesis Overview

This thesis consists of five chapters and the layout of the thesis is shown in Figure 1.1. Chapter one, titled motivation and design of the study, introduces the research overview, which includes the research problem, objectives, hypotheses, delineations of the research, significance of the study and expected outcomes. Chapter two is the literature review. A background on the nature, nutritional value and protein composition of soybean and BGN is given. The application of soybeans in the production of tofu and related products, as well as properties of tofu, are also outlined. Chapter three details the effect of gum Arabic and sodium alginate, as well as gluconolactone, lemon juice and vinegar, on the textural and physicochemical properties of

BGN tofu. The rheological behaviour of BGN milk extract is also discussed. Chapter four looks at the functional and consumer acceptability of BGN tofu chunks. Chapter five is the general summary, conclusions and recommendations.

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CHAPTER TWO
LITERATURE REVIEW

2.1 Tofu

Tofu is a nutritious, protein-rich bean curd derived from soybeans (Dey *et al.*, 2017). It originated in China and is widely consumed in East Asia. The production of tofu involves several steps: soaking, grinding and cooking soybeans, then separating the soymilk from the solid soy pulp or fibre (Yang *et al*., 2020; Wang *et al*., 2021). The soymilk is then coagulated by adding salt or acid, which causes the proteins to aggregate and form a gel-like structure. The coagulation step is the most important in the manufacture of tofu. Coagulants used in tofu production include salts, acids, enzymes and new alternatives such as emulsions and complex varieties (Dey *et al.*, 2017). The coagulation process in tofu production is influenced by protein subunits found in soybeans, particularly glycinin and β-conglycinin. These proteins, present in various types within soybeans, play a primary role in tofu coagulation (James & Yang, 2016; Guan *et al.*, 2021).

Glycinin and β-conglycinin, comprising approximately 70-80% of total soy protein, are soluble in water and have long chains of amino acids folded into complex 3D structures (Dey *et al.*, 2017; Sirison *et al.*, 2017). Each protein has a specific isoelectric point (pI), which is the pH at which it carries no net electrical charge and is least soluble in water (Chang *et al*., 2015; Mohanta *et al*., 2019). The pI of glycinin is around 4.5, while that of β-conglycinin is 5.2-5.5 (Krishnan *et al*., 2007). The pI of glycinin and β-conglycinin is important in the production of tofu as it affects the coagulation process and the formation of tofu curd. The association-dissociation reactions of these proteins during acid-base treatment and heat treatment change the ionic strength of the solution and affect the gelling properties of soy protein, which is essential for the formation of tofu. Tofu made from soybeans having high 11S/7S subunit ratios has a firm texture at lower coagulant concentrations than tofu produced from soybeans having low 11S/7S ratios (Krishnan *et al*., 2007; Onodera *et al*., 2009). According to Onodera *et al*. (2009), a high 11S/7S subunit (3:1) requires a high coagulant concentration (0.15-0.3%) to achieve maximum hardness. Achieving consistent quality and yield in tofu production is difficult due to various factors such as the type and concentration of coagulant, as well as the protein and lipid content of soybeans, which can affect the outcome. Therefore, additives are sometimes used in tofu production to modify the gel structure, texture, and processing parameters by interacting with soy proteins and enhancing curd formation.

The interaction between polysaccharides and protein polymers effectively improves the characteristics of the curd (Li *et al*., 2015; Chen *et al*., 2023). Hydrocolloids, such as guar gum, increase soymilk viscosity, resulting in a slower coagulation rate and a decrease in tofu hardness and protein content (Li *et al*., 2015). Conversely, carrageenan increases the hardness of the curd without affecting its protein content (Doddy *et al*., 2020). Chitosan, when used as a coagulant, reduces the ash content and improves the protein levels of tofu, while also enhancing its water-retention capabilities, especially in pressurised lactone tofu (Doddy *et al*., 2020). Dinani *et al*. (2023) demonstrated that incorporating hydrocolloids, such as albumins and globulins from mung beans, can enhance the interfacial and foaming properties of plant protein extracts. Pang *et al.* (2020) found that incorporating both a coagulant and a food hydrocolloid improves the gelation process and enhances the microstructure of soy protein gels. The amount of coagulant used influences the pore size, thickness and curvature of strands, which determine the gel properties (Pang *et al.*, 2020). Following a similar method of making tofu from soybeans, Bambara groundnut (BGN) tofu and its chunks was produced in this study.

2.1.1 Types of tofu

Tofu varieties are classified primarily by their water content and texture, categorised based on their softness or firmness (Yasin *et al.*, 2019; Dang *et al.*, 2023). The difference between firm and soft tofu is solely related to the amount of water content of the curd (Shurtleff & Aoyagi, 2016). The two major tofu types are silken tofu and block tofu. Silken tofu has a high moisture content and is un-pressed. It is made from coagulated extra-rich soymilk left to set so it becomes scoopable, custard-like, and jiggly. It is suited for saucy recipes, such as dressings, smoothies, and egg or yoghurt substitutions (Shurtleff & Aoyagi, 2016). Block tofu is pressed and has less water; hence it retains its shape better. This category includes super-firm, extra-firm, firm, medium, and soft tofu, each with varying water content and texture (Ali *et al*., 2021).

2.2 Effect of coagulants on the processing and quality of tofu

2.2.1 Type of coagulant

A coagulant is a compound or agent that promotes the aggregation of proteins by altering their charge distribution or solubility properties (Guan *et al*., 2021). Coagulants facilitate the formation of tofu by promoting interactions among proteins through changes in pH, ionic strength, or chemical bonding, leading to their coagulation and precipitation (Zeppa *et al*., 2021). These coagulants are categorised into three main groups, namely, acids, salts, and enzymes (Ingrid & Hanajaya, 2020).

Acids induce gelation of soy proteins through processes like isoelectric precipitation, which involve mechanisms such as salt bridging, hydrogen bonding and van der Waals forces (Cruz *et al*., 2023; Xu *et al*., 2019). Salts contribute to tofu formation by creating a three-dimensional network structure through salt bridges that link protein molecules. Specifically, compounds like phytic acids interact with Ca^{2+} ions to form non-ionised complexes, enabling interactions between Ca^{2+} ions and proteins. This interaction leads to the aggregation of proteins and the development of the gel network (Xu *et al*., 2019). Enzyme coagulants, as highlighted by Yang *et al*. (2021), facilitate protein assembly through the formation of isopeptide bonds between the amine group (glutamine residue) and the ξ-amino group (lysine residue), aiding in tofu formation.

Coagulants alter the pH of soymilk, which affects the charge distribution of the protein molecules (Darmajana *et al*., 2020). At the pI, proteins become neutral and are no longer repelled by charges, causing them to aggregate and precipitate out of solution (Darmajana *et al*., 2020). When the pH is adjusted to be slightly below the pI of soy proteins, they carry a net positive charge (Ingrid & Hananjaya, 2020; Darmajana *et al*., 2020). This causes repulsion between the protein molecules, preventing aggregation. When a coagulant is added, it reacts with the soy proteins, altering their charge distribution and causing them to aggregate and form a gel network, resulting in tofu formation (Darmajana *et al*., 2020). The effect of coagulants on tofu is significant in terms of altering its texture, water-holding capacity, and other properties. Different coagulants, such as calcium sulphate, nigari, and gluconolactone (GDL), create different structures in tofu by forming different types of protein gels (Xu *et al*., 2019; Ingrid & Hananjaya, 2020; Darmajana *et al*., 2020). The choice of coagulant used can influence the water-holding capacity of tofu gelatin, with an overall decreasing trend observed as coagulant concentration increases. The type of coagulant can also affect the textural properties of tofu.

Tofu made with Epsom salt, lemon juice and fermented maize water showed significant differences in hardness, chewiness and brittleness compared to calcium sulphate-coagulated tofu (Li *et al*., 2013; Zeppa *et al*., 2021). Protein gels are classified as either chemical or physical. Physical gels are mostly non-covalent networks, showing some frequency dependency and no crossover of G' (storage modulus) and G'' (loss modulus). Storage modulus refers to the elastic modulus of a material, which is a measure of its viscosity and elasticity and G" refers to the viscous or loss modulus of a material, which is a measure of the viscous portion of the viscoelastic response (Li *et al.*, 2023). Tofu prepared with salts has significantly higher G'. This is due to the structural changes induced by salt-induced protein interactions, resulting in a tofu product that is firmer and more resilient (Li *et al*., 2024). As such, the use of salts during tofu preparation enhances its firmness and elasticity as measured by the G'. The gelation rate at high salt concentrations is fast and results in the formation of large aggregates and a coarse structure of gel (Zeppa *et al*., 2021). Salts can improve the water structure and provide charge and polar groups, thus influencing electrostatic and hydrophobic interactions (Li *et al*., 2013).

2.2.2 Coagulant concentration

The concentration of coagulant plays a crucial role in influencing the protein content, mass, moisture, and texture of the final tofu product. Higher concentrations of coagulant produce firmer tofu, while lower concentrations produce softer tofu (Darmajana *et al*., 2020; Ingrid & Hananjaya, 2020). As the concentration of coagulant increases, tofu exhibits higher gel strength and reduced water retention. This effect arises from the coagulant induced denaturation of proteins in soymilk, leading to gel formation. Higher coagulant concentrations promote greater protein denaturation, resulting in a stronger gel and firmer texture (Zeppa *et al*., 2021). Ingrid & Hananjaya (2020) identified $CaSO_4$ and $MgSO_4$ as the most effective salt coagulants for tofu production. Specifically, the combination of $CaSO_4$ and GDL in a 1:1 ratio, along with a coagulation temperature of 70°C, yielded tofu with the highest protein content, mass, and improved texture.

2.2.3 Temperature and duration of coagulation

The effect of temperature and duration of coagulation on tofu production using coagulants is crucial for achieving the desired texture and quality of the final product. Higher temperatures and longer coagulation times can produce firmer tofu, while lower temperatures and shorter coagulation times can yield softer tofu (Zuo *et al.*, 2016; Guan *et al.*, 2021). Optimal coagulation temperatures play a significant role in determining the texture and quality characteristics of tofu. Coagulation below 70°C can result in tofu with a soft and runny texture, while coagulation above 85°C can result in hard and uneven tofu (Guan *et al*., 2021). Ideally, the coagulation process should be carried out within a temperature range of 70-85°C to achieve the desired consistency and texture of tofu (Ingrid & Hananjaya 2020).

2.2.4. Curd size

A higher concentration of coagulant yields larger curds due to the increased cross-linking of protein molecules, which leads to a more stable and firmer gel structure (Panthi *et al*., 2019). This is because a higher concentration of coagulant leads to a more rapid and complete precipitation of the proteins, resulting in larger curd sizes (Panthi *et al*., 2019; Guan *et al*., 2021; Huppertz & Chia, 2020). In contrast, a lower concentration of coagulant results in a smaller curd size due to the slower and less complete precipitation of the proteins, which leads to a weaker and less stable gel structure (Guan *et al.*, 2021). A lower concentration of coagulant allows for more protein unfolding and aggregation, resulting in smaller curd sizes (Guan *et al*., 2021). The size of the curd also affects the texture and rheological properties of the tofu (Panthi *et al*., 2019). Larger curd sizes result in a firmer, more elastic texture, while smaller curd sizes result in a softer, more delicate texture and a firmer gel structure (Panthi *et al*., 2019). The rheological properties of the tofu are also affected by the curd size, with larger curd sizes resulting in a more solid-like behaviour and smaller curd sizes resulting in a more liquid-like behaviour (Chen *et al*., 2023).

2.2.5 Tofu preparation

In the traditional Chinese method, soybeans are soaked in water overnight, ground with water, filtered and boiled for 10 min to make soymilk, then coagulated and pressed to make tofu (Huang *et al*., 2021). Pressing helps develop the desired consistency for soft, medium, or firm tofu. Removing excess water through pressing helps inhibit microbial growth and enzymatic activity, thus extending the shelf life of tofu (Rossi *et al*., 2016; Zhang *et al*., 2020). The traditional step-by-step process of soymilk and tofu production is shown in Figure 2.1.

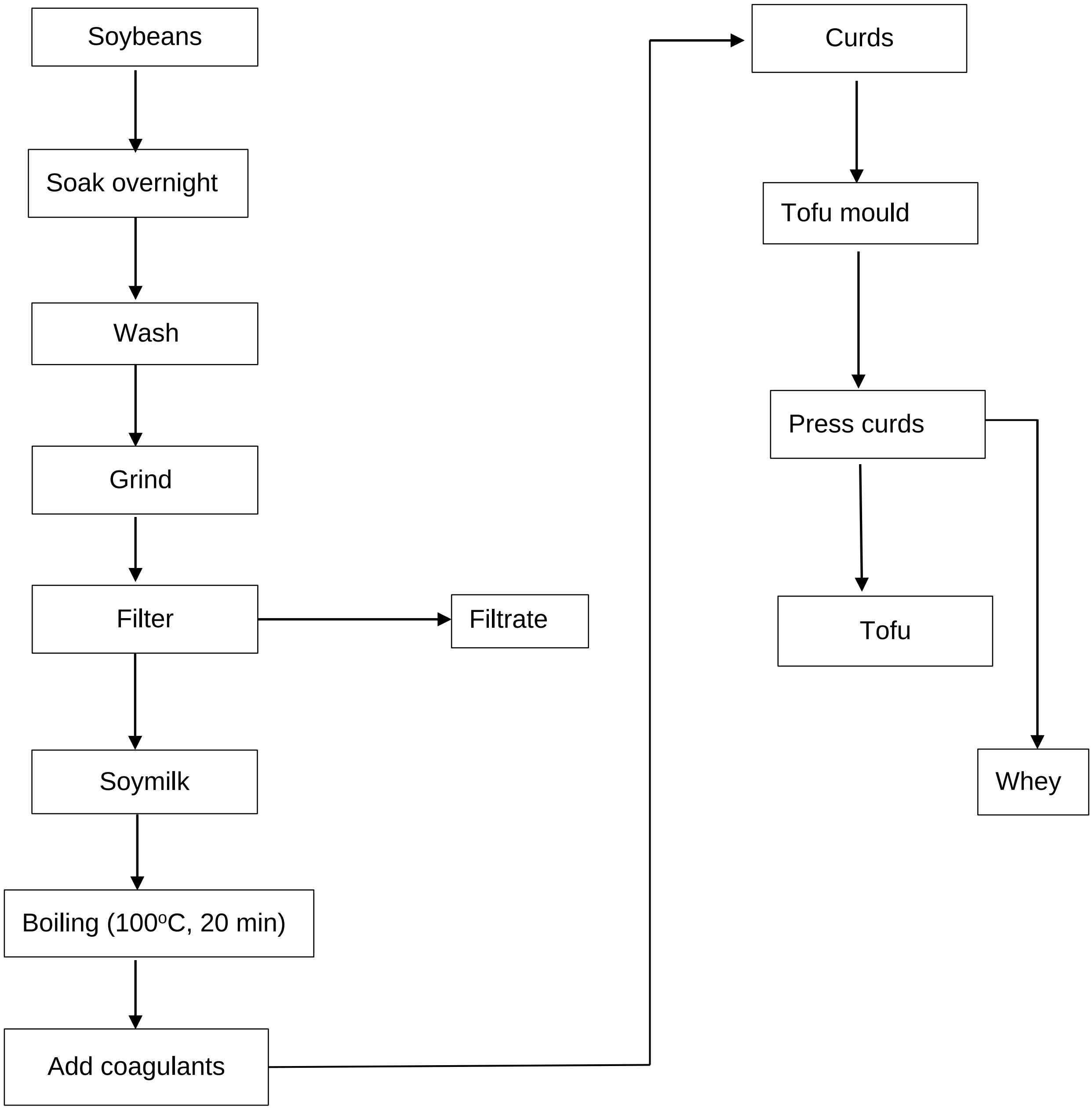

Figure 2.1: Process flow diagram of production of tofu from soybeans (Anon., 2018).

Tofu with lower water content is less prone to spoilage and can be stored for longer periods without significant deterioration in quality (Rossi *et al*., 2016). The protein content of whey contains leftover soy proteins that did not coagulate during the tofu-making process, as well as any proteins from the coagulant used (Chua & Liu, 2019). Whey also contains soluble vitamins and minerals from the soybeans, such as calcium, phosphorus, potassium, and various B vitamins. Additionally, whey can still be utilised for its nutritional value and incorporated into various food products. The removal of water allows the soy proteins to pack more tightly together, creating a cohesive and uniform structure. Therefore, pressing removes excess water (whey) from the tofu curds, resulting in a denser and firmer texture (Guan *et al*., 2021). Whey is the liquid by-product of tofu, consisting of water, along with dissolved proteins, carbohydrates, vitamins, minerals, and small amounts of fat (Chua & Liu, 2019).

Coagulation is a key step in tofu making, influenced by various factors. These factors, along with the percentage of solids in soymilk, are critical in determining the texture and yield of tofu. Curd formation is influenced by the interactions of protein particles, oil globules, and soluble proteins in soymilk when coagulants are introduced (Ono, 2010). Different coagulants yield tofu with distinctive textures (O'Toole, 2004). Additionally, factors such as the type and concentration of coagulants, coagulation temperature, and method of mixing the coagulant with soymilk affect the tofu coagulation process (Jayasena *et al*., 2014). The coagulation temperature is greatly affected by the type and concentration of the coagulant, method of coagulant addition, and desired type of tofu (O'Toole, 2004). As the temperature rises from 50 to 90°C, the yield of tofu decreases, with softer tofu at lower temperatures and harder, more uneven tofu at higher temperatures.

The process of making tofu relies on the gelation properties of soy protein. The fundamental principle involves extracting protein from soybeans by soaking them in water to produce soymilk, which is then coagulated using a coagulant. In general, the process of tofu production is divided into two main stages, namely, protein extraction and coagulation. The protein extraction method involves grinding soybeans, cooking soybean porridge, and filtering. The purpose of grinding soybeans is to break them down the soybeans into smaller particles, increasing their surface area for efficient protein extraction (Dzikunoo *et al*., 2015). It facilitates the release of soy proteins during the subsequent stages of cooking and filtering. Grinding soybeans aids the separation of soymilk from the solid soy pulp or fibre and is a crucial step in the tofu-making process (Dzikunoo *et al*., 2015). The efficiency of protein extraction is influenced by the grinding efficiency, as it affects the rate of protein mass transfer and solubility (Huang *et al*., 2021). After grinding, the soybeans are cooked to create a soybean porridge. Cooking the soybeans helps soften them further and prepares them for the extraction of soymilk (Zhang, 2020). This heating process denatures the soy protein and removes any volatile flavours, enhancing the quality of the final tofu product. The cooking process ensures the complete destruction of food pathogens and the inactivation of some bioactive substances such as lectins and trypsin inhibitors for food safety. Moreover, it also allows soy protein denaturation and aggregation to produce sol-gels, thereby contributing to the gelation process (Huang *et al*., 2021). Cooking at temperatures exceeding 120°C has been observed to increase the viscosity of soymilk, but it also leads to a significant decrease in soymilk quality due to excessive protein denaturation (Zuo *et al*., 2016). The use of high-temperature pressure cooking (HTPC) on soymilk increases the protein particle content (d > 40 nm), resulting in denser tofu that exhibits enhanced hardness, springiness, and chewiness (Zuo *et al*., 2016).

Filtering is a crucial step that helps to obtain smooth and consistent soymilk, which is essential for the coagulation process in tofu production. By filtering the soybean extract, the soymilk is separated from any solid particles, ensuring a clean and pure liquid that can be further processed for coagulation to form tofu (Darmajana *et al*., 2020). This step helps remove any unwanted particles or impurities, resulting in smooth and high-quality soymilk that is ready for the next stages of tofu production. Pressing tofu curds is a crucial step in the tofu-making process, and its significance lies in several factors that contribute to the texture, flavour, and shelf life of the final product (Guan *et al*., 2021).

2.3 Bambara groundnut

Bambara groundnut (*Vigna subterranea* (L) Verdc.) is an African indigenous grain legume that belongs to the family Leguminosae and it is mostly grown by subsistence farmers (Mabhaudhi & Modi, 2013). Bambara groundnut is the third most important legume after groundnut and cowpea in terms of consumption and socioeconomic impact in semi-arid Africa (Ibrahim *et al.*, 2018; Mayes *et al.*, 2019; Khan *et al.*, 2021). This legume has the potential to contribute to improved food and nutrition security while providing solutions for environmental sustainability and equity in food availability and affordability. It is recognised for its high nutritional value and is considered a complete crop (15–25% protein, 49–63.5% carbohydrate, 5.2–6.4% fibre, 4.5–7.4% fat, 3.2–4.4% ash and 2% mineral) (Jideani & Diedericks, 2014; Murevanhema & Jideani, 2014). Furthermore, BGN is tolerant to poor soils, drought, and salt stress and has the ability to yield in conditions where groundnuts/peanuts completely fail. The common names for BGN vary across different regions and cultures. In South Africa, other names for the BGN include izindhlubu (IsiZulu), hlanga (isiXhosa), nduhu (Venda), phonda (Tsonga) and tindhluwa (Tsonga) and jugo beans (English) (Khan *et al.*, 2021).

2.3.1 Varieties of Bambara groundnut

Bambara groundnut seeds come in different shapes, sizes and colours (Figure 2.2). The classification of BGN seeds is based on size, categorised as large (>10.5 mm), medium (9.50-10.49 mm), and small (<9.50 mm). The geometric mean diameter, volume, and surface area of these seeds vary between 9.59 and 9.98 mm, 435 to 498 mm^3, and 2608 to 2987 mm^2, respectively (Abu & Buah, 2011). Figure 2.2 shows mixed BGN seeds. A study by Ramatsetse *et al.* (2022) and Anon. (2011) identified seven types of BGN cultivars, namely black-eye, brown-eye, cream, red, black, brown and spotted. Among the seven cultivars, brown-eye and black-eye have the highest yield while cream yield has the lowest (Anon., 2011). Factors such as varieties and origin influence the chemical composition of BGN cultivars (Adeleke *et al*., 2017).

Figure 2.2: Different varieties of Bambara groundnut seeds (Chipeta & Jideani, 2017).

High temperatures and humidity can lead to the formation of lignin and pectin, making BGN take longer to cook (Ramatsetse *et al.*, 2023). Different seed colours and sizes can also impact the composition of BGN. For instance, light brown genotypes have been reported to have higher seed yields compared to other seed colours (Ramatsetse *et al.*, 2023).

2.3.2 Nutritional composition of Bambara groundnut

On average, a BGN seed is composed of 63% carbohydrates, 19-25% proteins, and 4.5-7.4% fat, with a fatty acid profile mainly consisting of linoleic, palmitic and linolenic acids (Abdua *et al*., 2012; Murevanhema & Jideani, 2014; Oyeyinka *et al.*, 2015). Bambara groundnut is considered a well-balanced food, rich in iron, with its protein containing high levels of lysine and methionine compared to many other food legumes (Tan *et al.*, 2020; Maphosa *et al.*, 2022). The proximate composition of BGN is shown in Table 2.1. The study of processing methods on the contents of anti-nutrients, oligosaccharides, and protein digestibility of BGN white (BGW) and BGN brown (BGB) flours highlighted protein content among different varieties of BGN seed. The protein contents of BGW (20.73%) and BGB (20.14%) were higher than the commercial (BGC) sample (18.50%) (Adeleke *et al*., 2017). Some of the key factors influencing the chemical composition of BGN cultivars could be environmental conditions such as temperature, humidity, and soil quality. A study by Adeleke *et al*. (2017) examined the impact of various processing methods on the anti-nutrient content, oligosaccharide levels, and protein digestibility of Bambara groundnut (BGN) flours: BGW and BGB.

Table 2.1 Proximate composition, minerals and phytic acid content of Bambara groundnut[1]

Component	g/100 g
Moisture	11.7 ± 0.1
Protein	18.8 ± 0.2
Fat	1.4 ± 0.3
Starch	50.2 ± 3.1
Amylose	17.6 ± 0.5
Sugars	2.4 ± 0.1
Total dietary fibre	10.3 ± 0.0
Soluble fibre	0.5 ± 0.2
Insoluble fibre	9.8 ± 0.2
Ash	2.9 ± 0.0
Calcium (Ca)	30.2 ± 1.6
Magnesium (Mg)	136.0 ± 2.0
Phosphorus (P)	33.35 ± 5.9
Iron (Fe)	8.8 ± 0.6
Copper (Cu)	0.5 ± 0.0
Zinc (Zn)	1.9 ± 0.1
Phytic Acid	1.1 ± 0.1

[1]Adapted from Yao *et al.* (2015), Huang *et al*. (2021).

The findings highlighted notable differences in protein content among the different BGN seed varieties. The protein contents of BGW (20.73%) and BGB (20.14%) were higher than the commercial (BGC) sample (18.50%). The chemical composition of the BGN cultivars was likely influenced by various environmental factors, such as temperature, humidity, and soil quality.

Carbohydrates make up the predominant macronutrient in BGN. The carbohydrate fraction is mainly composed of complex oligosaccharides and polysaccharides, with starch representing 22-49.5% (dry seed weight) of the total carbohydrates. Bambara groundnut starch primarily comprises amylose, amylopectin, water (15-20%), lipids (1%), proteins and minerals (Oyeyinka *et al*., 2018).

The protein content of BGN ranges from 19-25%, with an average value of 23.6%. The majority of BGN protein consists of storage proteins, with vicilin (7S) being the primary component, followed by legumin (11S) (Dey *et al*., 2017; Sirison *et al*., 2017). While high protein content is desirable in food, its quality is influenced by both amino acid composition and digestibility. Different cultivars of BGN show variations in amino acid profiles, with glutamic acid being the most abundant (Musah *et al*., 2021). Notably, essential amino acids like leucine and lysine are present in higher concentrations, whereas methionine is found in lower amounts (Musah *et al*., 2021; Adewumi *et al*., 2022). Phenylalanine, valine, histidine, and isoleucine are also reported to be present in significant concentrations (Musah *et al*., 2021; Adewumi *et al*., 2022).

The majority of fatty acids in BGN are unsaturated, predominated by oleic and linoleic acids (omega-6) (Ramatsetse *et al*., 2023). Palmitic acid is the third most abundant fatty acid, and linolenic acid (omega-3) is present at a low concentration. While having a high unsaturated fatty acid content is appealing from a consumer health perspective, it increases the susceptibility of fats to oxidation and rancidity. Therefore, the end use should be taken into consideration when selecting the desirable trait of lipid composition (Musah *et al*., 2021; Ramatsetse *et al*., 2023). Fatty acids play a crucial role as major components of cell membrane structure, gene transcription modulators, cytokine precursor functions, and energy sources within intricate interconnected systems (Glick & Fischer, 2013). The impact of dietary fatty acids on these essential functions is significant, influencing human health and potentially leading to cardiovascular diseases and mental health issues (Glick & Fischer, 2013). Fatty acids are classified into two groups, essential and non-essential, based on their synthesis capability and nutritional necessity. Essential fatty acids must be obtained from the diet, while non-essential fatty acids can be synthesised (Glick & Fischer, 2013; Insel *et al*., 2016).

Humans have the capacity to synthesise only 11 of the 20 amino acids required, while the remaining nine known as essential amino acids, must be sourced from basic foods. The amino acid contents and scores of protein in BGN are detailed in Table 2.2.

In their study on the fatty acid profile of BGN, Alhassanm *et al.* (2014) discovered a mix of saturated and unsaturated fatty acids with variations in chain length, double bond numbers and positions. The presence of arachidic acid (eicosanoic acid) suggests that BGN oil may be rich in essential fatty acids, particularly unsaturated fatty acids, which could play a significant role in cardiovascular health.

Roasted BGN seeds are recommended for treating polymenorrhea (Kone *et al*., 2011). Additionally, BGN is rich in soluble dietary fibre, believed to reduce the risk of heart disease and aid in preventing colon cancer (Kone *et al*., 2011).

Table 2.2 Amino acid composition of Bambara groundnut[1]

Amino acid	Crude protein (mg/g)	FAO Report Protein (mg/g)	AAS (%)
Histidine[1]	38.6 ± 2.3	16	2.41
Isoleucine[1]	54.5 ± 0.0	30	1.82
Leucine[1]	102.1 ± 0.1	61	1.67
Lysine[1]	80.2 ± 5.2	48	1.67
Threonine[1]	44.3 ± 3.2	25	1.77
Tryptophan[2]	6.0 ± 0.0	6.6	0.91
Valine[1]	62.4 ± 2.3	40	1.56
Methionine[1]	6.4 ± 0.1	-	-
Cysteine[2]	24.1 ± 0.0	-	-
Sulphur amino acid[2]	30.5 ± 0.0	23	1.33
Tyrosine[1]	31.3 ± 1.3	-	-
Phenylalanine[1]	76.9 ± 2.0	-	-
Aromatic amino acid[2]	108.2 ± 0.0	41	1.88
Aspartic acid[2]	146.1 ± 5.1	-	-
Serine[2]	68.5 ± 3.5	-	-
Glutamic acid[2]	209.5 ± 4.3	-	-
Proline[2]	53.6 ± 1.7	-	-
Glycine[2]	46.5 ± 3.4	-	-
Alanine[2]	51.4 ± 1.4	-	-
Arginine[2]	74.8 ± 6.0	-	-
AAS[3]	0.91 ± 0.0	-	-

[1]Essential amino acids. [2]Non-essential amino acids (Yao *et al*., 2015). AAS: [3]Amino acid scores. FAO: Food and Agriculture Organisation.

2.3.3 Uses of Bambara groundnut

Bambara groundnut is commonly used for household consumption as a dish or snack. Typically, the seeds are either pounded into flour and boiled to make a stiff porridge or soaked and then boiled for consumption (Tan *et al.*, 2020). Figure 2.3 shows various products made from BGN, including tofu, brownies, biscuits, sausages and tofu chunks.

Figure 2.3: Added value products produced from Bambara groundnut (Chipeta & Jideani, 2017).

Bambara groundnut flour affects the textural and leavening characteristics of various food products like ice cream, cakes, toppings, and confectionery items (Eltayeb *et al*., 2011). Recent studies have explored the potential of utilising BGN as a food ingredient. The absence of gluten in BGN flour negatively affects bread texture when added to the dough, limiting the amount that can substitute wheat flour. Research on incorporating BGN flour into bread production has shown effects such as protein weakening, prolonged dough development time, reduced dough consistency, stability, and extensibility, altered water absorption, and decreased loaf volume.

Bambara groundnut shows promise for producing vegetable milk and yoghurt. Reports have highlighted the development of shelf-stable spray-dried milk powder with favourable hydration properties. A study by Pahane *et al*. (2017) reported the nutritional and sensory attributes of BGN milk and yoghurt. The study highlighted that milk produced from BGN has a high nutrient content and produces a shelf-stable milk powder. In addition, Bambara groundnut yoghurt was low in pH during storage with an increased titratable acidity. However, products such as yoghurt and tofu made from BGN have not been commercialised yet.

Ali *et al*. (2021) conducted a study on processed flour with enhanced functional, nutritional, acceptability, and sensory attributes derived from BGN and cowpea seeds. The study emphasised the synergistic effect of BGN and cowpea flour when combined with wheat flour for biscuit production. This blend improved the essential amino acid composition and showed that processed flour from these legumes can replace up to 20% of cassava-based products. A successful new product of wheat bread made with BGN flour, as reported by Abdua *et al*. (2012), demonstrated enhanced protein quantity, *in-vitro* protein digestibility, and improved nutritional quality. This innovation in protein products has contributed to job creation, advancements in processing techniques, and the promotion of healthier dietary options.

The protein extracted from BGN can be utilised as a functional component in various food products such as baked goods, dairy and meat. Bambara groundnut protein isolate (BGPI) has a protein content ranging from 81.4 to 92.8% and exhibits solubility influenced by pH, surpassing that of mung bean and black bean protein isolates (Diedericks *et al.*, 2020a; Diedericks *et al.*, 2020b). Additionally, BGPI demonstrates notable thermal stability comparable to mung bean, black bean, and soy protein isolates, which is significant for applications in food processing and formulation (Diedericks *et al.*, 2020a; Diedericks *et al.*, 2020b).

2.3.4 Physicochemical properties of Bambara groundnut

Adebowale *et al*. (2011a) noted a lack of information on the preparation and properties of BGN protein isolates, which contributes to the underutilisation of BGN due to insufficient knowledge regarding its compositional analysis and potential uses. Starch, a primary energy source in cereals, legumes, and other foods, is a crucial component of BGN. The starch and flour from BGN have distinct structural and functional properties, characterised by a lower amylose content (21.7%) compared to that in cowpea (20-25%), soybean (15-25%) and chickpeas (25-30%) (Ma *et al.*, 2017; Maphosa *et al.*, 2022). Amylose is a key determinant of a favourable glycaemic response and resistant starch formation, offering health benefits related to glucose metabolism, energy intake, and colonic health. Starch is composed of glucose residues linked by α-1.4 and α-1.6 glucosidic bonds, with amylopectin and amylose as the main constituents of starch. Research has shown that starches isolated from BGN genotypes consist of predominantly oval-shaped granules, with varying amylose content (21–28%) depending on the source and variety (Kaptso *et al*., 2015; Oyenyinya *et al*., 2015). The amylose content significantly influences the functional properties of starch, including swelling, water absorption, paste clarity, and gelatinisation. Eliasson & Wahlgren (2004) highlighted that an overproduction of amylose increases the amylopectin chain length and phosphate content, further affecting starch functionality.

1. Gelling capacity of Bambara groundnut

The gelling property of legume proteins, such as those found in BGN, lies in their ability to form gels when subjected to heat (Ali *et al.*, 2021; Ma *et al.*, 2017). Heating the milk causes the dissociation of protein aggregates, exposing more hydrophobic regions and enhancing protein-protein interactions, leading to the formation of a stronger gel network (Ali *et al.*, 2021; Ma *et al.*, 2017). Gelation involves the unfolding of proteins, exposing reactive groups that lead to the aggregation of proteins into a three-dimensional gel network. The gelling property of any tofu is primarily determined by the composition and interactions of proteins, specifically the 11S (glycinin) and 7S (β-conglycinin) fractions. Proteins from BGN can form suitable gels for various food applications, including meat analogues, dairy-type products, and emulsifiers. The high protein content and balanced amino acid profile of BGN make it a potentially useful ingredient for developing functional food products, like meat substitutes and dairy alternatives, with desirable textural properties.

2. Foaming capacity of Bambara groundnut

The foaming properties of BGN protein isolates serve as a viable substitute for proteins in food applications that demand high foamability and stability, including cakes, ice cream, marshmallows, and desserts (Wang *et al.*, 2019). Foam can be defined as a two-phase system consisting of air cells separated by a thin continuous liquid layer called the lamellar phase. According to Eltayeb *et al.* (2011), foam can be produced by vigorously whipping air into liquid. Jain *et a*l. (2015) reported an increase in the foaming capacity and foam stability of BGN protein concentrate as compared to defatted groundnut flour and groundnut protein isolate. The flour produces foams due to the proteins in BGN flour being surface-active. The contact between air bubbles and the fluid that surrounds the medium can have surface tension decreased by soluble proteins (Diedericks *et al.*, 2020a; Yang et al., 2022). Therefore, the coalescence of the bubbles is prevented. In addition, protein molecules can unfold and interact with one another to form multilayer protein films with increased flexibility at the air-liquid interface. This results in air bubbles having difficulty breaking, which further stabilises the foam (Wang *et al.*, 2015; Diedericks *et al.*, 2020a).

3. Water and oil holding capacities of Bambara groundnut

The ability to retain water or oil against gravity through physical and physicochemical means is referred to as water-holding capacity and oil-binding capacity, respectively (Arise, 2016). The isoelectric precipitation of BGN proteins increases both water-holding and oil-binding capacity while membrane filtration decreases both (Jain *et al*., 2015). Arise (2016) reported that BGN protein concentrate prepared from salt solubilisation absorbed more water (2.4 mL g^{-1}) than those prepared via acid precipitation.

The water absorption capacity of BGN flour ranges from 0.51-1.12 g/g dry weight, which is less than the 1.6-2.8 g/g dry weight reported for roasted cowpea flour (Mashau *et al.*, 2022). The water-holding capacities of BGN dietary fibres vary depending on the cultivar. According to Maphosa & Jideani (2016), black-eye and brown-eye BGN fibre varieties have higher water-holding capacities (2.84 and 2.83 g water/g sample, respectively) compared to the brown and red varieties (2.60 and 2.41 g water/g sample, respectively). This variability in water-holding capacity could be beneficial for various food applications.

Figure 2.4 shows the formation of droplets within the T-junction, with the dispersed phase represented by the smaller droplets and the continuous phase being the larger, surrounding fluid. The interfacial region is the area where the dispersed and continuous phases meet, characterised by a diffuse interface description (Chirkov *et al.*, 2024).

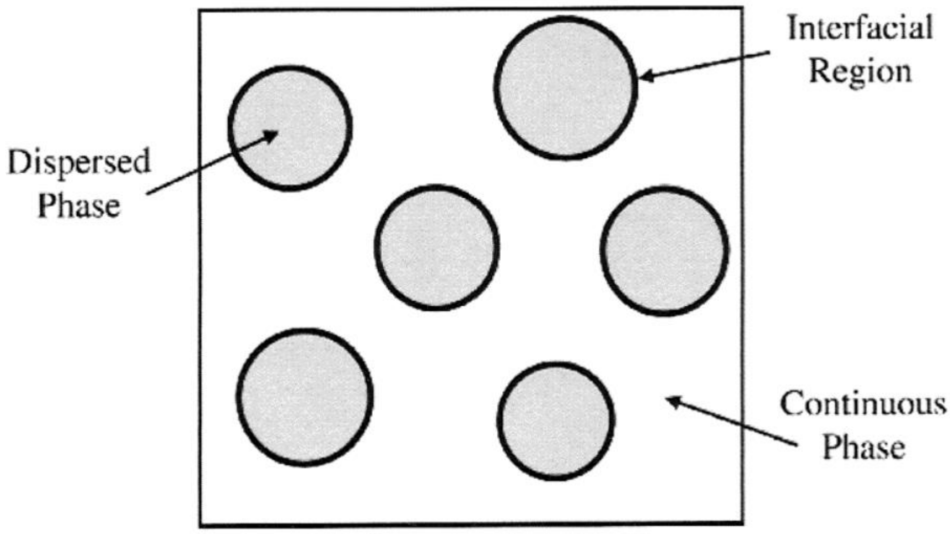

Figure 2.4: Interfacial region in a dispersed and continuous system (Chirkov *et al.*, 2024).

2.4 Bambara groundnut storage protein

Storage proteins from abundant protein sources like soybeans have been utilised in food applications to enhance functionality. The glycinin (11S) and β-conglycinin (7S) protein fractions are known to directly impact the textural properties of tofu due to their structural and property differences (William & Akiko, 2000). Glycinin, with a lower isoelectric point (pI), tends to aggregate and precipitate more readily at slightly acidic pH levels, forming the structure of the tofu gel (William & Akiko, 2000). Studies by William & Akiko (2000) and Guo & Yang (2015) have shown that tofu made from crude 11S protein is firmer than tofu made from 7S protein. The crude 11S fraction contributes to the springiness, chewiness, and gumminess of tofu, while the 7S fraction requires more calcium or magnesium for coagulation (O'Toole, 2004; Adebowale *et al*., 2011a). Research by Adebowale *et al*. (2011b) indicates that BGN protein isolates are not dissociated by 1,4-Dithiothreitol (DTT), suggesting the absence of subunits linked by disulphide bonds, implying that 7S may be the primary storage protein in BGN isolates. In soymilk, the 11S fraction, the α′ polypeptide of 7S, and the basic polypeptide of 11S each influence tofu yield but are not correlated with tofu firmness. However, tofu firmness is related to the ratio of the 11S fraction, the 7S fraction, and their ratio (O'Toole, 2004). These major components of soy proteins exhibit distinct gelation characteristics, with significant hydrophilic groups like glycan and extension regions present in both, but not in 11S globulin. The aggregation of 7S globulin is comparatively limited when compared to that of 11S globulin. Guo & Yang (2015) observed that gels formed by 11S globulin with various coagulants like calcium ion, GDL, and heat treatment differ in their properties.

2.5 Rheology

Rheology is the study of the deformation of solids and the fluidity of liquids influenced by the applied mechanical forces (Bonfim & Pereira, 2017). There are two main types of fluids in rheology: Newtonian and non-Newtonian fluids. The Newtonian fluid has a viscosity that is independent of the rate of deformation applied (Samaee *et al.*, 2022). On the other hand, the non-Newtonian fluid viscosity depends on the rate of deformation which may or may not depend on shear time. Thus, food rheology parameters are directly related to the acceptance of the final product by consumers. The rheological behaviour of food, such as dairy products, is influenced by factors such as processing, product stability, and organoleptic properties (Samaee *et al*., 2022). For these reasons, food rheology is an important analytical tool for evaluating food quality. Rheological characterisation is done to measure the functional connection between stress and deformation (Isendhal, 2022).

Milk and cream exhibit Newtonian or non-Newtonian behaviour depending on the composition, conditions and processes to which they are subjected (Morrison *et al*., 2013; Huang *et al*., 2021). The composition of milk is essential in determining the rheological characteristics of dairy products (Huang *et al*., 2021). The rheological properties of milk, such as viscosity, can be modified by adding hydrocolloids (Quintana *et al*., 2022). This allows control over the coagulation rate and adjusting the textural and compositional properties of the resulting tofu. Optimising the rheological behaviour of milk is important for producing consistent, high-quality tofu (Huang & Kuo, 2015).

Bambara groundnut milk has a relatively high viscosity because of its high protein content and the presence of complex carbohydrates (Murevanhema & Jideani, 2014). This higher viscosity can affect the coagulation rate and final texture of tofu and other products made from BGN milk (Ruzengwe, 2021).

2.6 Hydrocolloids in tofu production

Hydrocolloids are colloidal substances that exhibit a strong affinity for water (Gao *et al*., 2024; Zang *et al*., 2024). They are macromolecular, hydrophilic compounds that can be either dissolved in water to form colloidal solutions or swell in water and disperse under shear forces (Zang *et al*., 2024). Hydrocolloids can produce viscous solutions, pseudo-gels, or gels in water. Hydrocolloids are commonly utilised in food products because of their functional properties, which encompass thickening, gelling, emulsification, stabilising foam, preventing the formation of ice and sugar crystals, and enabling controlled flavour release.

Hydrocolloids are commonly utilised in food products because of their functional properties, which encompass thickening, gelling, emulsification, stabilising foam, preventing the formation of ice and sugar crystals, and enabling controlled flavours release (Gao *et al*., 2024). The water-thickening property is common among all hydrocolloids, making it a primary reason for their use.

While all hydrocolloids are biopolymers as a result of their natural origin and molecular structure, not all biopolymers exhibit the specific water-binding and thickening properties characteristic of hydrocolloids (Hu *et al*., 2024). A biopolymer is a large molecule composed of repeating units called monomers, which are naturally derived from living organisms (Baranwal *et al*., 2022). These polymers can be proteins, polysaccharides (such as starches, cellulose, and gums), or nucleic acids (like DNA and RNA).

Biopolymers serve diverse roles in food beyond thickening, such as providing structure, texture, and nutritional value, whereas hydrocolloids are primarily valued for their ability to modify the rheological properties of food systems, enhancing stability and sensory attributes (Baranwal *et al*., 2022; Hu *et al*., 2024).

In food systems, gelling biopolymers can be classified into two main categories: proteins and polysaccharides (Li *et al*., 2023). Understanding the mechanisms underlying the interactions between proteins and polysaccharides is crucial for exploring their potential in creating novel gel textures. Hundschell *&* Wagemans (2019) reported that making the liquid phase more viscous, uncharged polysaccharides support the stability of protein gel systems. Ionic polysaccharides bind electrostatically with positively charged areas of the protein surface that have lower charge densities when the pH is lower than the protein's isoelectric point (pI) (Krishnan *et al*., 2007).

The texture of tofu can be modified by varying the hydrocolloid concentration, counter-ion type or concentration, and by blending with different carrageenan types, as well as by mixing with other hydrocolloids in various ratios (Shen & Kuo, 2017). Previous studies have identified carrageenan, gum Arabic and corn starch as key ingredients for improving the textural properties of tofu (Shen & Kuo, 2017; Murad *et al*., 2015). The addition of carrageenan enhances its localised coagulation with protein aggregates, leading to tofu with a coarser network and firmer texture.

2.7 Plant-based protein sources in meat alternatives

An increasing number of people are showing interest in vegetarian or vegan diets, prompted by concerns over the environmental impact of meat production and the nutritional benefits of plant-based foods. Both vegetarian and vegan diets have been linked with numerous health benefits, such as reduced body mass index (BMI) values, reduced chance of developing chronic illnesses such as diabetes, heart disease, and cancers, and improved overall health (Wang *et al*., 2023). Vegetarian diets are often chosen to increase awareness about animal cruelty and slaughter for food. Some individuals choose vegetarianism for ethical, religious and environmental reasons (Wang *et al*., 2023). The market for meat alternatives is experiencing significant growth, including products derived from traditional protein sources such as soy and gluten, as well as innovative sources such as peas, fava beans, and BGN (Kurek *et al*., 2022; Imran & Liyan, 2023).

Meat-like alternatives can be produced from various sources of protein other than soybeans, including legumes, cereals, oilseeds, algae, insects, and fungi (Kyriakopoulo *et al*., 2021). Legumes such as lupin seeds and fava beans are alternative protein sources that can be used in meat-like products (Kyriakopoulo *et al*., 2021; Lima *et al*., 2022). Plant-based mince-like alternatives such as those produced from soybeans, cowpeas and BGN provides a sustainable and eco-friendly substitute for common meat products (Langyan *et al*., 2022; Singh *et al*., 2024).

Animal agriculture is a major contributor to greenhouse gas emissions, deforestation, and land degradation (Xu *et al*., 2021). In contrast, plant-based protein sources have a lower environmental impact and can help reduce the carbon footprint of the food system (Gibbs & Cappucio, 2022). Plant-based meat alternatives provide a healthy and nutritious source of protein for consumers (Singh *et al*., 2024).

Soybean is a rich source of high-quality protein, dietary fibre, vitamins, and minerals and is low in saturated fat and cholesterol, making it a healthier alternative to traditional meat products (Mazumder *et al*., 2023). According to a report by the World Wildlife Fund (WWF), 75% of global soy output is used in animal feed, while only 25% is used for human consumption, biofuels, and other industrial purposes (Thrane *et al*., 2017).

Soy mince is a widely recognised and highly nutritious plant-based protein source. Soybeans are a complete protein source containing all of the essential amino acids with a digestibility score equivalent to meat and dairy foods (Karabulut *et al*., 2024). Anjum *et al.* (2011) stated that major development has occurred in the texturisation of plant proteins in the food industry. Furthermore, depending on the chemical composition of proteins and the properties of individual components, legumes, especially soybeans, are valuable for the production of texturised vegetable proteins (TVP), primarily through the extrusion process.

2.8 Tofu extrusion

Texturising is done using high temperature, pressure and shear forces on proteinous and non-proteinous constituents while maintaining a limited amount of excess water in the extruder (Rajendra *et al*., 2022). Extrusion technology is a versatile and efficient method of converting raw materials into finished food products. Its advantages include energy efficiency, lack of process effluents and versatility with respect to ingredient selection and the shapes and textures of products to be produced (Penaranda *et al*., 2023).

Extrusion has the ability to alter proteins (from both plant and animal sources), starches, and other food materials to create a range of novel and distinctive food products (Rajendra et al., 2022). (Rajendra *et al*., 2022). In addition, because extrusion is a high-temperature short-time (HTST) heating method, it reduces the degradation of food nutrients while enhancing the digestibility of proteins (through denaturation) and starches (through gelatinisation) (Penaranda *et al*., 2023). Interestingly, researchers are exploring BGN tofu as a novel basic ingredient for mince production, marking its first use in this application. Bambara groundnut protein curd has demonstrated potential in extrusion using juice extraction equipment. Figure 2.5 shows extruded BGN protein that resembles a mince-meat like-product.

Figure 2.5: Bambara groundnut protein curd extrusion.

Textured vegetable proteins are plant-based products designed to entirely replace meat in a food or to complement meat as extenders (Rajendra *et al*., 2022). Textured vegetable protein simulates meat in both chewiness and flavour. Overall, the appearance, taste, texture, aroma, and mouthfeel of real meat is essential to the success of plant-based meat substitutes. However, because meat substitutes have different technological needs and require texturisation for improvement, making vegetable protein-based products might be challenging. During the extrusion of Bambara protein curd, notable issues with stickiness or clumping of the curds were observed, indicating a need for process modifications.

2.9. Alternative legumes for making tofu

Bambara groundnut is a promising alternative legume for producing high-protein products due to its high protein content (15–25%) (Tan *et al*., 2020; Diedericks, 2020b; Murevanhema & Jideani, 2014). Furthermore, BGN has a rich nutritional profile and bioactive components.

Similar to BGN, sweet lupin (*Lupinus angustifolius* L.) is an Australian legume crop with one of the highest natural protein and dietary fibre contents, as well as a low level of anti-nutritional factors. This combination enhances the bioavailability of its protein and nutrients, eliminating the requirement for soaking and extensive cooking (El-hadidi, 2017). Sweet lupin, unlike other legumes, can be eaten fresh, hence enhancing its versatility. In Australia, sweet lupin flour and flakes are used in baked goods such as gluten-free bread, cookies and cakes, as well as uncooked smoothies and dips. Sweet lupin contains about twice the amount of protein contained in widely consumed legumes (Conejo *et al*., 2023). Because of the characteristics of growing conditions and soil types, there are differences in protein content (28-48%) among species and cultivars. In many foods, sweet lupin has proven to be a beneficial substitute for soybeans. Furthermore, sweet lupin contains a low fat content (6% compared to 18% of soybeans) and a higher dietary fibre content (30% compared to 9% of soybeans), making it a better option for use in a variety of foods (El-hadidi, 2017). Sweet lupin has nutritional and functional properties that are similar to those of soybean, and it can be used to substitute soybean in a variety of foods, including tofu. Sweet lupin has a lower fat content than soybean, but its protein content is equivalent. The fat content of tofu can be decreased without affecting its sensory acceptability by using sweet lupin. In addition, tofu that is lower in fat but higher in protein would be a healthier alternative to regular tofu and may be a good fit for low-fat, high-protein diets. Since lupin is almost half the price of soybeans, integrating it into tofu could result in significant cost savings (El-hadidi, 2017).

Cowpea (*Vigna unguiculata* L. Walp) is also considered a healthy alternative to soybean as consumers seek more traditional food sources that are low in fat, high in fibre and offer other health benefits (Shalimar, 2023). Cowpeas typically contain carbohydrates (50-65%), protein (20-30%), fat (2.71-2.96%) and an adequate amount of minerals and vitamins (Shalimar, 2023). Cowpeas are a good source of lysine and tryptophan but deficient in cysteine and methionine. The introduction of a more practical method for preparing cowpea products such as tofu provides an alternative source of protein. This innovation meets the increasing global demand for protein and aligns with dietary recommendations. Legumes can be processed into meat analogues that resemble meat in appearance, texture and flavour with minimal effort on the part of consumers. In this study, tofu and tofu chunks made from BGN were successfully produced, demonstrating excellent physicochemical properties and satisfactory sensory qualities.

2.10 Conclusions

The high protein composition of BGN makes it a promising alternative protein source, offering the potential to reduce reliance on crops like soybean and cowpea, which are commonly used in the food industry for tofu and its chunk production. By incorporating BGN into these processes, the pressure on traditional protein sources (soybeans and cowpeas) would be alleviated, promoting crop diversity and sustainability while providing a nutritious, plant-based alternative that meets the increasing global demand for high-quality protein products. This would also increase the market value of BGN and result in its role in lessening malnutrition and increasing food security. The inclusion of gum Arabic and sodium alginate during the making of tofu not only modifies its textural and water absorption properties but also positively impacts the nutritional composition and sensory qualities of tofu chunks. Studying the functionality of gum Arabic and sodium alginate on the textural, rheological, and physicochemical properties of BGN tofu and tofu chunks is essential for predicting their optimal levels in order to produce consumer-acceptable tofu chunks.

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CHAPTER THREE
EFFECT OF HYDROCOLLOIDS ON THE TEXTURAL, RHEOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF BAMBARA GROUNDNUT TOFU

Abstract

Bambara groundnut (BGN) (*Vigna subterranea* [L.] Verdc.) is a source of protein (15-25%), making this legume a suitable alternative to soybean for tofu production. The use of BGN as a main ingredient in tofu is not documented, as most tofu is made from soy milk extracted from soaked soybeans. The texture (Instron texture analyser), colour (HunterLab ColorFlex spectrophotometer), rheology (rheometer), proximate (AOAC methods) and microstructure (scanning electron microscope) of BGN tofu as affected by coagulants (vinegar, lemon juice and gluconolactone (GDL)) and hydrocolloids (gum Arabic and sodium alginate) were established in this chapter. Factorial design and response surface methodology were used to optimise gum Arabic and sodium alginate concentrations at 0.3, 0.4 and 0.5%. The interaction of gum Arabic and sodium alginate was optimal at 0.5%. The texture of tofu with gum Arabic and sodium alginate ranged from 9.11 to 9.15% (hardness), 11.45 to 11.46% (springiness), and 19.72 to 21.94% (gumminess). The combination of gum Arabic and sodium alginate significantly ($p < 0.05$) increased the hardness and springiness of tofu and decreased its gumminess. The lightness of BGN tofu ranged from 54.83 (vinegar-tofu) to 64.00 (lemon juice-tofu). The lightness of tofu produced with 0.6% GDL, 1% GDL and lemon juice did not differ significantly. An increase in tofu lightness was observed with increasing GDL concentrations. The coefficient of determination (R^2) for all measurements with the Power law was >0.9. The consistency coefficient (K) of BGN milk extract prepared using GDL with gum Arabic and sodium alginate was >1, indicating shear-thickening behaviour. The effect of coagulants on the consistency coefficient (K), flow behaviour (n) and coefficient of determination (R^2) of BGN tofu was significant ($p < 0.001$). The expressible and entrapped water content of BGN tofu ranged from 17.13 (0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate) to 27.27% (1% GDL-tofu) and 45.13 (1% GDL-tofu) to 65.60% (0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate), respectively. Adding gum Arabic and sodium alginate to BGN tofu increased the protein, ash and carbohydrate content and decreased the moisture content. Vinegar-tofu exhibited a uniform, smaller particle size network and smooth surface, while 0.6% GDL-tofu and 0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate had larger particle size networks and a coarse texture. Coagulant type, concentration, and hydrocolloids significantly influenced the texture, physicochemical properties and rheological behaviour of BGN tofu. Gum Arabic and sodium alginate could significantly improve the quality, shelf life and overall acceptability of BGN tofu and related products.

3.1 Introduction

The ability of hydrocolloids to alter the rheological attributes of systems is the main reason for their use in food products. Hydrocolloids are a heterogeneous group of long-chain polymers (polysaccharides and proteins) that form viscous dispersions and gels (Schwenzfeier *et al.*, 2014; Sharma *et al.*, 2017; Exarhopoulos *et al.*, 2018). They form an intermediate dispersion between a real solution and a suspension with colloid-like features. Water-thickening is a characteristic common to all hydrocolloids and is the primary reason for their widespread use (Solano *et al.*, 2022; Ekonomou *et al.*, 2024). Hydrocolloids disperse in water, imparting a thickening or viscosity-producing effect, with most achieving high viscosities at concentrations below 1% (Ekonomou *et al.*, 2024). The degree of thickening depends on the kind and nature of the hydrocolloid. Changes in the texture and viscosity of food modify its sensory qualities. Hydrocolloids find use in foods such as meat and meat-based products.

According to Samard *et al.* (2021), meat emulsion products balance quality and quantity requirements related to functionality and nutritional value, resulting in an effective gel with a protein-water and protein-protein balance. Few biopolymers can also form gels. Gel formation entails the association or cross-linking of polymer chains to create a three-dimensional network that traps or immobilises the water within it to form a rigid structure that is resistant to flow (Panyathitipong & Puechkamut, 2010; Zheng *et al.*, 2021). Thus, the system becomes viscoelastic, exhibiting both liquid and solid features. Soy protein and hydrocolloids, such as carrageenan and gum Arabic, have been utilised in meat emulsion products, and when combined, they improve processing and quality features (Lorenzo *et al*., 2015). Gum Arabic (Acacia gum) is widely used as an emulsifier in beverage emulsions to impart desirable qualities because of its influence over viscosity, body and texture. The gum has a low-viscosity that imparts viscosity at 30% concentration compared to 1% xanthan or carboxymethylcellulose (CMC) at low shear rates. Saha & Bhattacharya (2010) and Gao *et al.* (2017) discovered that dispersions of gum Arabic (4-50%, w/v) exhibit shear-thinning behaviour at low shear rates (10 s^{-1}) and Newtonian plateaus at shear rates above 100 s^{-1}. Gelling agents such as sodium alginate are commonly used in meat-based products.

Sodium alginate is used as a texturiser in meat to retain water and improve the elasticity, stickiness and freshness of finished products. It achieves this by complexing with calcium ions forming a calcium alginate gel that traps water in the meat. Sodium alginate finds use in products like tofu.

Tofu is a plant-based protein-rich food commonly produced from soybeans. It is a highly digestible, water-based protein gel containing all the essential amino acids required by the human body (Okpuzor *et al*., 2011; Nadathur *et al*., 2017). Its production involves blending soaked soybeans, filtering, boiling and coagulating soy milk, moulding and pressing the curd to release whey. Energy increases as raw soy milk is heated, causing the thermal motion of protein molecules to become more intense and the vibration frequency of some groups in the molecules to rise. The secondary bonds that hold the protein molecular structure together break due to this change, resulting in spatial structural changes. Hydrophobic groups such as sulfhydryl groups, disulfide bonds, and hydrophobic amino acid side chains are exposed during the thermal denaturation of soy proteins, leading to an increase in their hydrophobicity, thereby intensifying protein molecule aggregation (Ringgenberg *et al.*, 2013; Khoder *et al.*, 2020; Wang *et al.*, 2014). The soy-protein gel is the basis for the formation of tofu. The amount of water needed to prepare soy milk is crucial as it alters the soy milk solid content and the quality and texture of the resultant tofu. Reduced soy milk solids result in a higher moisture content, thus producing soft-textured tofu (Kamizake *et al.,* 2016). Producing tofu with consistent quality and yield is difficult as many factors influence the results. As such, additives are typically used to modify the textural characteristics of tofu.

Bambara groundnut is a highly nutritious crop, with high protein (15-25%), carbohydrate (63%), fat (6.5%) and dietary fibre (5.5%), making it a 'complete food'. This legume has the potential to provide essential nutrients and improve the nutritional needs of food-insecure households. Adebowale *et al.* (2011) and Gupta *et al.* (2018) indicated that the protein content of BGN isolates performs well when compared with soybean, obtaining 90 and 70% by isoelectric precipitation or micellisation, respectively, while soybean contains 92 and 72%, respectively. Additionally, BGN protein isolates are not dissociated by dithiothreitol (DDT) and are not linked by disulphide bonds. Vicilin (7S) and legumin (11S) are the main components of BGN protein, making up a significant portion of the protein content in BGN, ranging from 81.4 to 92.8%. The major components of soybean tofu curd are β-conglycinin (7S) and glycinin (11S), which account for more than 70% of the total soy protein composition (James & Yang, 2016; Guan *et al.*, 2021). As such, BGN shows potential as a functional food ingredient (Adebowale *et al.*, 2011; Gupta *et al.*, 2018; Diedericks *et al.*, 2019) for tofu. The growing demand for plant-based protein-rich foods that contribute to food security, sustainability and a healthy diet, necessitates the need for new, inexpensive and easily accessible sources of protein.

While technological and functional properties of BGN protein have been extensively studied, there is limited research on its functionality, textural and rheological properties, as well as the impact of processing parameters on the yield of BGN tofu. This study aimed to establish the textural, physicochemical and rheological properties of BGN tofu as affected by coagulants (gluconolactone (GDL), vinegar and lemon juice) and hydrocolloids (gum Arabic and sodium alginate).

3.2 Materials and Methods

3.2.1 Source of materials and equipment

Bambara groundnuts were purchased from Triotrade, Johannesburg, South Africa. Gum Arabic, sodium alginate and GDL were purchased from Sigma-Adrich (Pty) Ltd, South Africa.

Food-grade coagulants (vinegar and lemon juice) were purchased from a local supplier (Checkers, South Africa). All other materials and equipment were obtained from the Department of Food Science and Technology of the Cape Peninsula University of Technology, Bellville.

3.2.2 Production of Bambara groundnut flour

Whole BGN seeds were screened and sorted to eliminate defective seeds and physical hazards such as stones. The seeds were washed and dried at 50°C for 48 h in a cabinet dehydrator (Excalibur, Model No: EXC10, NSF, USA) then milled using a hammer mill (Three-Phase Induction Motor, Frame T1CR 160M-4, S/No: 10091585) with a 250 µm sieve. Bambara groundnut flour (BGNF) was stored in plastic zip lock bags and kept in the refrigerator at 4°C before use.

3.2.3 Production of Bambara groundnut tofu

Bambara groundnut milk was produced by blending BGNF with water (1:10 w/v) at room temperature (25°C ± 1) for 5 min using a food mixer (Kenwood 7-qt major stand mixer). The mixture was allowed to hydrate undisturbed for 2 h, followed by filtration through a cheesecloth three times. It was then brought to a boil at 100ºC for 15 min (Chipeta & Jideani, 2017). From a preliminary experiment by Chipeta & Jideani (2017), 4% vinegar and 4% lemon juice were determined to produce the optimum BGN tofu. The coagulation process was initiated separately, by adding vinegar (4%), lemon juice (4%) and gluconolactone [GDL] (0.3, 0.6, 1%). Gluconolactone (0.3, 0.6, 1%) was compared with vinegar (4%) and lemon juice (4%). Protein curds from each coagulant were then transferred into a polypropylene mould (18 cm x 11 cm x 12 cm) and covered with a cheesecloth, pressed for 24 h at room temperature using a 5 kg rectangular cast iron weight to obtain the tofu. To determine the optimum GDL concentration, BGN tofu was analysed for textural and colour properties. The optimum BGN tofu was used to study the effect of gum Arabic and sodium alginate on the tofu.

3.2.4 Effect of coagulants on textural properties of Bambara groundnut tofu

A texture analyser (Instron, System ID Number 3344K6233, Serial Number: 63470, Model: 251-106, USA) equipped with a flat compression plate probe (S/No: s15909) was used to measure the textural properties (hardness, springiness, and gumminess) of BGN tofu following a modified method adapted from Syah *et al.* (2015); Zuo *et al.* (2016); Palanisamy *et al.* (2018) and Yang *et al.* (2020). Cubic-shaped samples (1.9 x 1.9 x 1.9 cm) were prepared from the central part of the tofu and each sample was loaded at the centre of the Instron stand. A compression test consisting of first and second bite was performed from 80 and 20% compression of the original sample height at a constant speed of 50 mm/min. Each assessment was carried out in triplicate and the results of hardness, gumminess and springiness were recorded automatically on the Bluehills software, United Kingdom.

3.2.5 Effect of coagulants on colour properties of Bambara groundnut tofu

The methods of Chavan *et al.* (2017); El-hadidi (2017); Zhao *et al.* (2020) and Li *et al.* (2021) were used to describe the surface colour of BGN tofu. The ColorFlex (HunterLab, S/N CX1668, Illuminant D65 10° Observer) spectrophotometer was first calibrated by placing white and black standard tiles on the instrument. BGN tofu (20 g) was transferred into the glass sample cup and covered with a black lid. Lightness (L*), redness/greenness (a*) and yellowness/blueness (b*) were assessed using L*C*h* and CIE-L*a*b* colour space systems. Measurements were carried out in triplicate.

3.2.6 Numerical optimisation of Bambara groundnut tofu with hydrocolloids

A response surface I-optimal point exchange randomised design was used to determine the effect of gum Arabic (0.3, 0.4, 0.5%) and sodium alginate (0.3, 0.4, 0.5%) on the textural, physicochemical and rheological properties of BGN tofu. The design consisted of six models, with three lack of fit and three replicate points, giving a total of twelve runs as shown in Table 3.1. The appropriate amount (Table 3.1) of gum Arabic and sodium alginate was added to boiling BGN milk as described in section 3.2.3. The mixture was stirred and then coagulated individually by adding vinegar (4%), lemon juice (4%) or gluconolactone (GDL) (0.3, 0.6, 1%).

Table 3.1 I-optimal point exchange randomised response surface design for the effect of gum Arabic and sodium alginate on Bambara groundnut tofu[1]

	Independent variables	
Run	**Gum Arabic**	**Sodium alginate**
1	0.4	0.4
2	0.4	0.5
3	0.4	0.3
4	0.5	0.3
5	0.5	0.4
6	0.4	0.4
7	0.3	0.3
8	0.3	0.4
9	0.4	0.4
10	0.3	0.5
11	0.4	0.4
12	0.5	0.5

Each coagulum was then transferred into a polypropylene mould and covered with a cheesecloth. The tofu was obtained by pressing the coagulum for 24 h at room temperature using a 5 kg rectangular cast iron weight. To determine the optimum hydrocolloid concentration, BGN tofu was analysed for textural properties as described in section 3.2.4 and colour properties as described in section 3.2.5. A second-order polynomial regression model (Equation 3.1) was used to estimate the linear, interaction and quadratic effects of the variables.

$$Y = \beta_0 + \beta_1 X_1 + \beta_1 X_2 + \beta_1 \beta_2 X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (3.1)$$

Where Y is the response variable; X_1 and X_2 are the main effects of gum Arabic and sodium alginate, respectively; X_1X_2 is the interaction effects of gum Arabic and sodium alginate; X_1^2 and X_2^2 are quadratic terms of gum Arabic and sodium alginate, respectively; β_0 is the model coefficient estimate for the Y intercept and β_1, β_2, β_{11} and β_{22} are the regression coefficients for the main, interaction and quadratic effects respectively.

Model adequacy was assessed using coefficient of determination (R^2), lack fit and adequate precision (signal to noise ratio). The graphic three-dimensional response surface plots were generated to show the effect of gum Arabic and sodium alginate on the texture and colour of BGN tofu. Numerical optimisation to maximise hardness, springiness and minimise gumminess was used to estimate the optimum combination of the independent variables for optimum BGN tofu (Design-Expert software, 2023, version 23.0).

3.2.7 Effect of hydrocolloids on textural properties of Bambara groundnut tofu

The compression behaviour of BGN tofu coagulated with GDL was evaluated using the method described in section 3.2.4. Images of tofu produced with and without hydrocolloids before and after undergoing compression were captured and described.

3.2.8 Determination of expressible water and entrapped water in Bambara groundnut tofu

The expressible water in BGN tofu was determined using the modified methods of Liu *et al*. (2013); Shen & Kuo (2017) and Khoder *et al.* (2020). A small piece of cotton wool was weighed and placed at the bottom of a centrifuge tube to retain expressed liquid. Tofu (10 g) was transferred into the centrifuge tube and centrifuged (Jouan, Centrifuge MR 18-12, Thermo Electron Corporation in France) at 2500 x *g* for 60 min at 4°C. The weight of the cotton wool was re-recorded after centrifugation to obtain the value of the expressed water. The expressible water was calculated as the percentage of the expressed liquid in the original tofu (Equation 3.2). Entrapped water was calculated by subtracting the expressible water from the original water content of the tofu sample (Equation 3.3). Measurements were carried out in triplicate.

$$Expessible\ water\ = \left(\frac{Weight\ of\ expressed\ fluid}{Weight\ of\ original\ sample}\right) \times 100 \quad (3.2)$$

$$Entrapped\ water\ = (Total\ water\ content\ - Expressible\ water) \quad (3.3)$$

3.2.9 Proximate analysis of Bambara groundnut tofu

The proximate composition of BGN tofu was determined using standard Association of Official Analytical Chemists International (AOAC) methods (Cassini *et al*., 2006). Moisture and total ash were determined using the vacuum oven method (AOAC method 934.01 and 923.03) (Murdia & Wadhwani, 2014) and the protein content was determined using the Kjeldahl method, calculated as nitrogen multiplied by a factor of 6.25 (N X 6.25). The fat content was measured using the Soxhlet method (AOAC method 996.06), a semi-continuous solvent extraction approach. Carbohydrates were quantified using the difference method where the sum of the percentage of moisture, ash, protein and fat was subtracted from 100%. Each analysis was carried out in triplicate.

3.2.10 Microstructure of Bambara groundnut tofu

A scanning electron microscope (SEM) was used to analyse the network structure of BGN tofu using a method adapted from Zheng *et al.* (2021). Tofu samples were cut into small pieces and fixed in 2.5 M glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 4 h at 4°C. The samples were rinsed with the same buffer, freeze-dried for 24 h and then coated with gold. The images were obtained at 2000X magnification. All measurements were carried out in triplicate.

3.2.11 Effect of hydrocolloids on the rheological properties of Bambara groundnut milk extract

Samples of BGN milk extract, obtained as described in section 3.2.3, were used in the evaluation of the steady shear rheological properties of BGN milk with and without gum Arabic and sodium alginate using a rheometer (RheolabQC C- PTD 180/AIR/QC, Anton Paar, S/No: 81602927, Austria). The method followed was adapted from Agoda-Tandjawa *et al.* (2017) and Jo *et al.* (2018). Samples were taken immediately after cooling and each sample was transferred into the rheometer cup (4 cm diameter). The shear rate and shear stress data were obtained over a shear rate range of 0.1 to 1000 s^{-1} at 25°C and fitted to the Power law model (Equation .3.4) to describe the flow properties. To investigate the effect of temperature on viscosity, steady shear rheological measurements were carried out across temperatures ranging from 15 to 25°C. Measurements were carried out in triplicate.

$$\tau = Ky^n \quad (3.4)$$

Where τ is the shear stress (Pa), K is the consistency index (Pa.s^n), n is the flow behaviour index (dimensionless) and y is the shear rate (s^{-1}).

3.2.12 Data analysis

IBM Statistical Package for the Social Sciences (SPSS) version 27 (2020) was used for statistical analysis (Ezenwa & Iheme, 2022). All experiments were carried out in triplicate and results were expressed as mean ± standard deviation. Multivariate analysis of variance (MANOVA) was used to establish differences between treatments. Duncan's multiple range test was used to separate means where significant ($p \leq 0.05$) differences existed.

3.3 Results and Discussion

The tofus coagulated with 4% lemon juice, 4% vinegar, 0.3% GDL, 0.6% GDL and 1% GDL were referred to as lemon juice-tofu, vinegar-tofu, 0.3% GDL-tofu, 0.6% GDL-tofu and 1% GDL tofu, respectively.

3.3.1 Effect of coagulants on textural properties of Bambara groundnut tofu

The textural properties of BGN tofu prepared with different coagulants varied as shown in Table 3.2. The maximum peak force during the first compression cycle (first bite) is known as hardness (Chavan *et al.*, 2017; Zheng *et al.*, 2021). Tofu hardness is influenced by pressing (Yuan & Chang, 2007). As a result, in this study, a weight of 5 kg was used to press the tofu curds for 24 h. Hardness is significantly influenced by the water content within the tofu matrix. The more water trapped in the matrix, the lower the hardness. The hardness of BGN tofu ranged from 1.96 N (0.3% GDL-tofu) to 6.73 N (1% GDL-tofu).

Table 3.2 Effect of coagulant concentration on the textural properties of Bambara groundnut tofu[1]

Coagulant	Hardness (N)	Springiness (mm)	Gumminess (N)	Springiness (%)
4% Lemon juice	3.04 ± 1.04^{a,b}	11.07 ± 0.14^{a}	28.55 ± 8.32^{a}	88.54 ± 1.07^{a}
4% Vinegar	4.14 ± 1.52^{b,c}	11.14 ± 0.07^{a}	42.85 ± 16.28^{a,b}	89.17 ± 0.53^{a,b}
0.3% GDL	1.96 ± 0.56^{a}	11.25 ± 0.16^{a}	22.52 ± 5.04^{a}	90.78 ± 1.29^{a}
0.6% GDL	5.78 ± 1.13^{c,d}	11.21 ± 0.07^{a}	64.39 ± 17.45^{b}	89.66 ± 0.77^{a,b}
1% GDL	6.73 ± 1.23^{d}	11.23 ± 0.04^{a}	64.94 ± 24.78^{b}	89.84 ± 0.27^{a,b}

[1] Values are mean ± standard deviation of triplicate analysis. Means within a column followed by the same superscripts are not significantly ($p > 0.05$) different. [2]GDL: Gluconolactone.

Type of coagulants had a significant ($p \leq 0.05$) effect on the hardness of BGN tofu. There was no significant difference in hardness between vinegar-tofu, lemon juice-tofu and tofu coagulated with 0.3 GDL. Furthermore, there was no significant difference between 0.6% GDL-tofu and 1% GDL-tofu, while both significantly ($p < 0.05$) differed from 0.3% GDL-tofu. Incorporating GDL has been reported to assist in the production of soft and compact tofu (Jayasena *et al.*, 2014; Kamizake *et al.*, 2016; Assalam *et al.*, 2020; Inggrid & Hananjaya, 2020). Similarity in hardness between lemon juice-tofu and 0.3% GDL-tofu can be attributed to the fact that acidic coagulants, such as lemon juice and GDL, at low concentrations yield soft tofu with a fragile texture due to a decrease in pH and high moisture content (Gartaula, *et al*., 2014; TRes *et al.*, 2019; Ezeama & Dobson, 2019). According to Arise *et al.* (2017), using GDL coagulants reduces milk pH, resulting in the aggregation of denatured proteins by increasing their hydrophobic nature. Dey (2017) reported that slow gelation and lower coagulation concentration of 7S proteins result in a soft tofu, while higher coagulation concentration and faster gelation of 11S proteins result in a hard tofu. In this study, an increase in tofu hardness was observed with increasing GDL concentration. This was in agreement with Yanti *et al*. (2021), who reported an increasing hardness in Jack bean tofu with increasing GDL concentration, from 0.25% GDL (4.23 N) to 0.5% GDL-tofu (5.10 N) to 1% GDL-tofu (5.53 N).

Springiness refers to how effectively a product regains its original shape after deformation during the initial compression. It is a measure of the product's elasticity and ability to recover its shape and structure, thus affecting mouthfeel and consumer acceptability. Low springiness indicates that the product has low elasticity, which may make it less bouncy and less desirable in terms of texture (Nishinari *et al*., 2014). The springiness of BGN tofu ranged from 11.07 mm (lemon juice-tofu) to 11.25 mm (0.3% GDL-tofu). There was no significant difference in the springiness of all tofu variants. The springiness of tofu did not change significantly with the type and concentration of coagulant. However, all the tofu coagulated with GDL were springier than those coagulated with lemon juice and vinegar. According to Kurrat & Pekins (2020), GDL form a slower, more controlled coagulation process that creates a tighter and more elastic protein network.

Gumminess is the energy required to disintegrate semisolid food into a state ready for swallowing (Yuan & Chang, 2007; Hwang *et al*., 2012). As chewing begins, food is compressed, sheared and torn, causing it to disintegrate in preparation for swallowing. As such, gumminess is a measure of the strength of the cohesive and adhesive forces within the food, and is related to the protein content, structure and processing conditions of the food (Rosenthal & Thompson, 2020). The gumminess of BGN tofu ranged from 28.55 N (lemon juice-tofu) to 64.94 N (1% GDL-tofu). There was no significant difference between lemon juice-tofu, vinegar-tofu and 0.3% GDL-tofu. Furthermore, there was no significant difference between 0.6% GDL-tofu and 1% GDL-tofu, while both differed significantly ($p < 0.05$) from 0.3% GDL-tofu. The texture of tofu is significantly affected by the composition and ratio of storage proteins (Wu *et al.*, 2016; Wang *et al.*, 2020). The main storage proteins in BGN are vicilin (7S) and legumin (11S), with vicilin (53 kDa) being responsible for gelation and hardness (Diedericks *et al*., 2019). A lower (32.7%) 11S/7S ratio in soy protein gel results in uniform spherical aggregated gels, while a higher (92.6%) ratio leads to higher macroscopic phase separation and a coarser network structure (Wu *et al*., 2016).

The type of coagulant also affects the protein fraction that coagulates in tofu (Jayasena *et al.*, 2014). Low concentrations of acidic coagulants, such as vinegar or lemon juice, produce softer tofu due to higher moisture content, while higher concentrations of coagulants like GDL increase hardness. As GDL concentration increases, so does tofu hardness, because of the formation of a fibrous structure that enhances gumminess, chewiness, and springiness. The increase in hardness is attributed to aggregation through hydrophobic interactions, thereby inducing gelation, strengthening the protein network and firming the tofu texture (Guan *et al.*, 2021).

Lemon juice was not used for further analysis due to the formation of small curds. Instead, vinegar (4%) and GDL (0.3, 0.6,1%) were used for further analyses.

3.3.2 Effect of coagulants on Bambara groundnut tofu colour

The colour characteristics of BGN tofu were described in terms of lightness (L*), redness/greenness (a*) and yellowness/blueness (b*) (Table 3.3). Lightness describes the brightness or darkness of a colour, measured on a scale of 0 (black) to 100 (white) (Maphosa, 2016). The red or green component of a colour is indicated by positive or negative values of a*, respectively, while the yellow or blue component is indicated by positive or negative values of b*, respectively. A value of 0 for either a* or b* indicates a neutral colour (Maphosa, 2016). The lightness of BGN tofu ranged from 54.83 to 64.02, where vinegar-tofu was the darkest and lemon juice-tofu was the lightest. Lemon juice-tofu had the lightest colour, which could be attributed to the bleaching properties of citric acid present in lemon juice (Karim *et al.*, 2022).

Table 3.3 The effect of coagulant concentration on Bambara groundnut tofu colour[1]

	Colour parameter		
Coagulant	**Lightness (L*)**	**Redness/Greenness (a*)**	**Yellowness/Blueness (b*)**
4% Lemon juice	64.02 ± 1.40[a]	2.63 ± 2.61[a,b]	6.53 ± 0.88[a]
4% Vinegar	54.83 ± 0.98[b]	5.24 ± 0.84[b]	4.48 ± 0.55[a,b]
0.3% GDL	55.33 ± 0.90[b]	2.37 ± 1.32[a]	3.29 ± 1.58[b]
0.6% GDL	62.18 ± 0.77[a]	3.45 ± 0.60[a,b]	6.57 ± 2.24[a]
1% GDL	64.00 ± 0.69[a]	3.71 ± 0.73[a,b]	6.73 ± 1.57[a]

[1]Mean values of triplicate determinations ± standard deviation. Means within a column followed by the same superscripts are not significantly ($p > 0.05$) different. [2]GDL: Gluconolactone.

The variation in tofu colour may be attributed to the different bleaching properties of the coagulants. A higher L* value is desirable since consumers prefer lighter or whiter tofu (Lee & Jung 2017). The use of coagulants significantly ($p < 0.05$) lightened BGN tofu. The lightness of 0.6% GDL-tofu, 1% GDL-tofu and lemon juice-tofu did not differ significantly from each other but were all significantly ($p < 0.05$) lighter than vinegar-tofu and 0.3% GDL-tofu (Table 3.3). An increase in lightness was observed with increasing GDL concentration.

The redness/greenness of BGN tofu ranged from 2.37 to 5.24 for 0.3% GDL-tofu and vinegar-tofu, respectively. Although vinegar-tofu was the reddest, it did not differ significantly compared with lemon juice-tofu, 0.6% GDL-tofu and 1% GDL-tofu.

The yellowness/blueness of BGN tofu ranged from 3.29 to 6.73 for 0.3% GDL-tofu and 1% GDL-tofu, respectively. Gluconolactone concentration did not significantly affect the redness/greenness of tofu. The 0.6% GDL-tofu and 1% GDL-tofu were more yellow than 0.3% GDL-tofu and did not differ significantly from lemon juice-tofu and vinegar-tofu in yellowness. There was a noticeable difference in the yellowness of 0.3% GDL-tofu compared to lemon juice-tofu, 0.6% GDL-tofu and 1% GDL-tofu. Although lemon juice-tofu was lighter than other samples, it was not used for further analysis due to the formation of small curds during coagulation. Small curds delay production as more time is needed to remove excess whey.

3.3.3 Numerical optimisation of hydrocolloid concentration effects on textural properties of Bambara groundnut tofu.

The objective of numerical optimisation in this chapter was to determine the optimum combination of gum Arabic and sodium alginate to produce a tofu with the maximum hardness and springiness, and minimum gumminess. Only the texture parameters were modelled. Vinegar was used as the coagulant in this experiment. The effect of gum Arabic and sodium alginate on the texture (hardness, springiness and gumminess) of BGN tofu is described in this section.

1. Model adequacy

The descriptive statistics for the effect of gum Arabic and sodium alginate on the textural properties of BGN tofu are detailed in Table 3.4. The data were fitted to a quadratic polynomial model and the effects of the independent variables and the model parameters are reported in Table 3.5. In terms of hardness, the quadratic model was significant ($p < 0.05$), with a high R^2 (0.9978), low coefficient of variation (1.84%), an insignificant lack of fit (0.4610) and a desirable adequate precision (58.7956). The coefficient of determination (R^2) is a relative measure of goodness of fit between experimental data and predicted model data. Adequate precision measures the signal to noise ratio and was greater than 4 (Table 3.5) implying it was desirable. The high R^2 and adequate precision indicated that the quadratic model could be accurately used to predict the effect of gum Arabic and sodium alginate on the hardness of BGN tofu. However, for springiness and gumminess, the linear model with insignificant lack of fit, desirable adequate precision of greater than 4, and high R^2 could be used to navigate the design space. The overall model showed significant effects on hardness ($p < 0.0003^*$), springiness ($p < 0.0183^*$), and gumminess ($p < 0.0190^*$). This suggests that the model effectively explains the variability in tofu texture properties based on the studied variables (gum Arabic, sodium alginate, and their interactions) and adequately fits the data.

2. Effect of hydrocolloid on the textural properties of Bambara groundnut tofu

From Table 3.5, the primary effect of gum Arabic, the interaction between gum Arabic and sodium alginate and the quadratic effects of gum Arabic and sodium alginate significantly ($p < 0.05$) affected the tofu hardness. The predicted relationship between the hardness and the hydrocolloid is illustrated in equation 3.5 and the response surface is shown in Figure 3.1.

$$\text{Hardness} = 7.40 + 1.22\,X_1 - 0.0250\,X_2 + 1.68\,X_1X_2 - 0.5385\,X_1^2 - 0.5803\,X_1^2 \qquad (3.5)$$

Where X_1 is gum Arabic and X_2 is sodium alginate.

The main effect of gum Arabic significantly increased the hardness of BGN tofu while sodium alginate increased it by 1.22 units. As the amount of alginate increased, the hardness of the tofu slightly decreased by 0.025 units assuming all factors remain constant. Furthermore, the interaction effect of gum Arabic and sodium alginate was synergistic resulting in increased BGN tofu hardness more than the sum of their individual effects. This means that the combined effect of both hydrocolloids was greater than their individual contributions. The quadratic effects represent the curvature in the response surface due to each hydrocolloid. The negative coefficients for the quadratic effects of gum Arabic and sodium alginate show that there is a diminishing return effect as the amounts of gum Arabic and sodium alginate increase, resulting in a decrease in the rate of increase in hardness and eventually leading to a decrease if either hydrocolloid becomes too large. This means that the effect of gum Arabic and sodium alginate on the hardness of tofu is not linear and there are optimal levels for maximising the hardness. Hydrocolloids like gum Arabic and sodium alginate are studied for their ability to modify tofu texture by interacting with water and soy proteins. Gum Arabic has been found to increase tofu hardness by improving water retention and forming a more cohesive gel network (Nikhil & Pradip, 2020). Conversely, sodium alginate imparts a softer texture by enhancing water binding and reducing protein aggregation. The interaction between these hydrocolloids and other ingredients further complicates the texture profile, with studies suggesting synergistic effects when used in combination. Overall, research on tofu hardness underscores the multifaceted nature of texture modification in tofu production, influenced by a combination of formulation, processing conditions, and ingredient interactions (Lou & Zhang, 2021).

Table 3.4 Effect of gum Arabic and sodium alginate concentration on the textural properties of Bambara groundnut tofu[1]

	Independent variables		Texture			
Run	**Gum Arabic (%)**	**Sodium alginate (%)**	**Hardness (N)**	**Springiness (mm)**	**Gumminess (N)**	**Springiness (%)**
1	0.3	0.3	6.84 ± 2.75	11.22 ± 0.07	102.98 ± 3.60	89.76 ± 0.62
2	0.3	0.4	5.57 ± 0.83	11.31 ± 0.07	66.73 ± 4.78	90.51 ± 0.50
3	0.3	0.5	3.33 ± 0.27	11.28 ± 0.10	39.65 ± 2.96	90.26 ± 0.82
4	0.4	0.3	6.72 ± 0.60	11.40 ± 0.05	71.72 ± 6.75	91.22 ± 0.36
5	0.4	0.4	7.37 ± 2.93	11.34 ± 0.09	73.63 ± 32.48	90.71 ± 0.71
6	0.4	0.4	7.37 ± 2.93	11.34 ± 0.09	73.63 ± 32.48	90.71 ± 0.71
7	0.4	0.4	7.37 ± 2.93	11.34 ± 0.09	73.63 ± 32.48	90.71 ± 0.71
8	0.4	0.4	7.37 ± 2.93	11.34 ± 0.09	73.63 ± 32.48	90.71 ± 0.71
9	0.4	0.5	6.88 ± 0.49	11.38 ± 0.10	64.72 ± 17.80	91.04 ± 0.86
10	0.5	0.3	5.89 ± 0.99	11.37 ± 0.07	82.11 ± 17.21	90.98 ± 0.54
11	0.5	0.4	6.04 ± 0.29	11.38 ± 0.02	46.66 ± 4.98	91.04 ± 0.11
12	0.5	0.5	9.11 ± 2.74	11.53 ± 0.35	142.11 ± 16.08	90.79 ± 0.30

[1]Mean values of triplicate determinations ± standard deviation.

Table 3.5 Model parameters for the effect of gum Arabic and sodium alginate on the textural properties of Bambara groundnut tofu[1]

	p-value		
Source	**Hardness (N)**	**Springiness (mm)**	**Gumminess (N)**
Model	< 0.0003*	< 0.0183*	< 0.0190*
Gum Arabic	< 0.0002*	< 0.0091*	0.1375
Sodium alginate	0.6477	0.1977	0.0069*
Gum Arabic * Sodium alginate	0.0001*		
Gum Arabic^2	0.0087*		
Sodium alginate^2	0.0085*		
Model adequacy			
Lack of fit	0.4610	0.6592	0.9020
R^2	0.9978	0.5888	0.7332
Adjusted R^2	0.9942	0.4974	0.6442
C.V. %	1.84	0.4998	19.23
Adequate precision	58.7956	7.6756	7.7277

[1]*Significant at $p \leq 0.05$. R^2: coefficient of determination. C.V: coefficient of variation.

The BGN tofu produced with the highest amount (0.5%) of gum Arabic and highest amount (0.5%) of sodium alginate had the maximum hardness.

The springiness of BGN tofu ranged from 11.22 mm [Run 1 (0.3% gum Arabic:0.3 sodium alginate)] to 11.53 mm [Run 12 (0.5 gum Arabic:0.5 sodium alginate)] as shown in Table 3.4. From the linear model (Table 3.5) it was observed that the main effect of gum Arabic was significant ($p < 0.05$) on the springiness of BGN tofu, while sodium alginate did not significantly affect the springiness of BGN tofu. Increasing the concentration of gum Arabic significantly ($p < 0.05$) increased the springiness of tofu. However, the effect of sodium alginate was not significant (Equation 3.6, Figure 3.2). It can be concluded that gum Arabic had a more substantial impact on increasing springiness compared to sodium alginate. This relationship helps to understand how different levels of the hydrocolloids affect the springiness of tofu and can be used to adjust the formulation to achieve the desired texture.

$$\text{Springiness} = 11.35 + 0.0767X_1 + 0.0322\,X_2 \quad (3.6)$$

Where X_1 is gum Arabic and X_2 is sodium alginate.

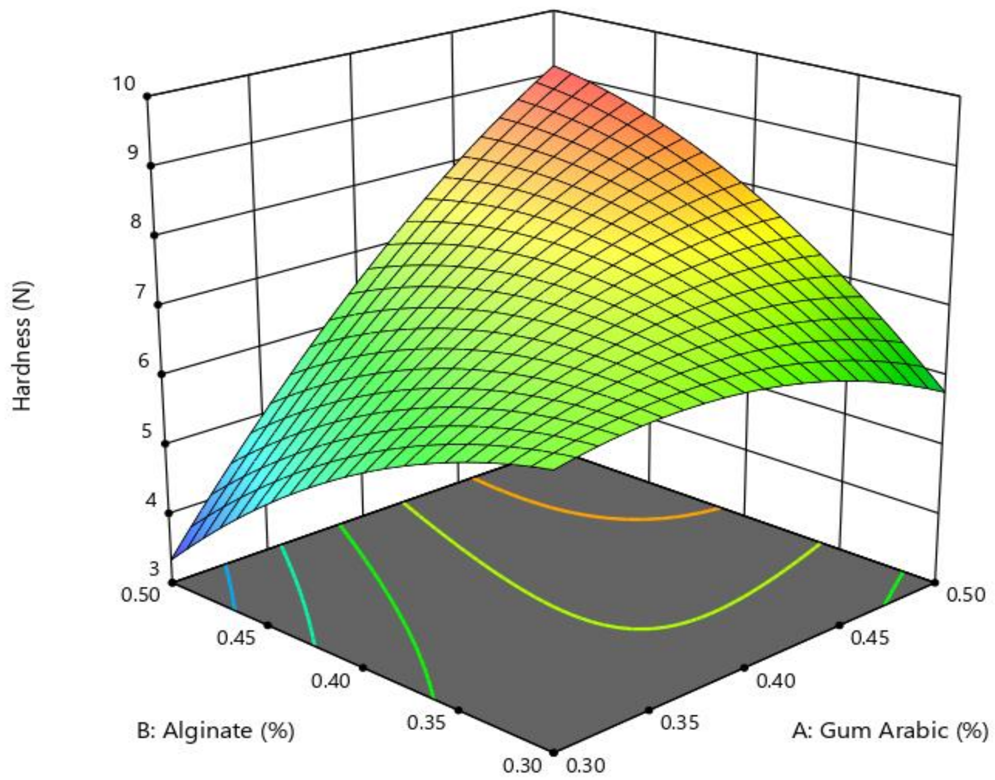


Figure 3.1 Effect of gum Arabic and sodium alginate on Bambara groundnut tofu hardness.

Equation 3.6 describes the effect of gum Arabic and sodium alginate on tofu springiness. The constant term (11.35) represented the baseline springiness of tofu when both gum Arabic and sodium alginate were zero. The coefficient of X_1 (0.0767) indicated that increasing the concentration of gum Arabic slightly increased tofu springiness. Similarly, the coefficient of X_2 (0.0322) suggested that higher concentrations of sodium alginate also contributed to a modest increase in tofu springiness. This equation (Equation 3.6) indicated that both gum Arabic and sodium alginate improved the springiness of tofu by influencing its water retention and gel formation properties, which enhanced its ability to recover its original shape after deformation. The relationship between gum Arabic and sodium alginate concentrations and tofu springiness was examined to understand their combined effect on the ability of tofu to regain its initial form after compression. Higher concentrations of both gum Arabic and sodium alginate generally corresponded to slightly higher springiness values across different experimental runs. For instance, at fixed gum Arabic levels (e.g., 0.3% or 0.4%), increasing sodium alginate from 0.3% to 0.5% showed minor increases in springiness. Similarly, at fixed sodium alginate levels (e.g., 0.3% or 0.4%), increasing gum Arabic concentration from 0.3% to 0.5% also demonstrated a trend towards increased springiness. This indicated that both hydrocolloids contributed positively to tofu springiness by enhancing water retention and gel formation properties that improved the elasticity of tofu. The interaction effect between gum Arabic and sodium alginate

(X_1X_2) further supported this trend, indicating that their combined presence at higher concentrations synergistically enhanced tofu's ability to maintain its springy texture. Overall, these findings highlighted the interplay between gum Arabic and sodium alginate in influencing tofu springiness, which was crucial for optimising texture properties in tofu production.

The BGN tofu produced with the highest amount (0.5%) of gum Arabic and the highest amount (0.5%) of sodium alginate had the maximum springiness.

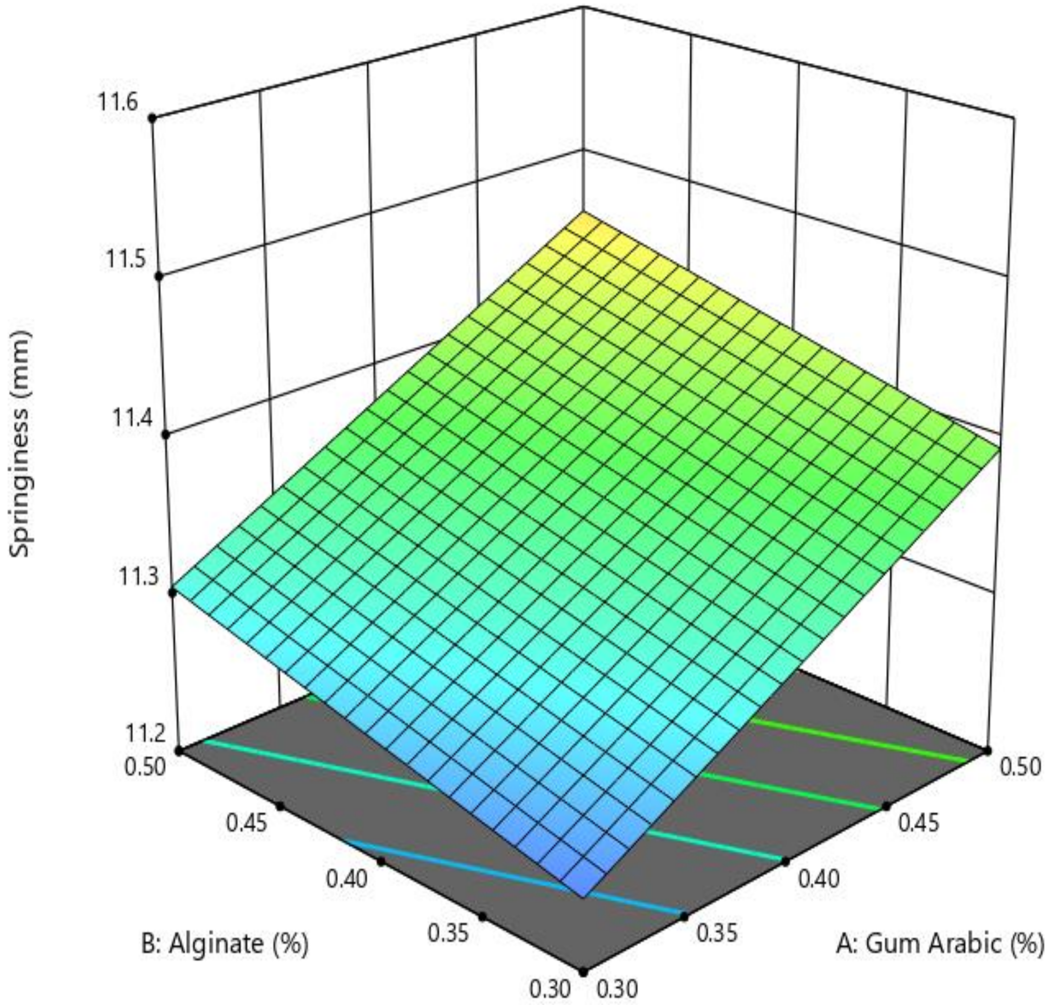


Figure 3.2: Effect of gum Arabic and sodium alginate on Bambara groundnut tofu springiness.

The gumminess of tofu ranged from 39.65 N [Run 3 (0.3 gum Arabic:0.5 sodium alginate)] to 142.11 N [Run 12 (0.5 gum Arabic:0.5 sodium alginate)], as shown in Table 3.4.

From Table 3.5, main effects of gum Arabic and sodium alginate had a linear effect on tofu gumminess. While increasing the sodium alginate produced a significant ($p < 0.05$) effect, that of gum Arabic was insignificant. The predicted relationship between the gumminess and the hydrocolloid is detailed in Equation 3.3.7 and the response surface is presented in Figure 3.3.

$$\text{Gummine ss} = 57.78 - 10.15\, X_1 - 27.94\, X_2 \qquad (3.7)$$

Where X_1 is gum Arabic and X_2 is sodium alginate.

Equation 3.7 describes the effect of gum Arabic (X_1) and sodium alginate (X_2) on tofu gumminess. The constant term (57.78) represents the baseline gumminess of tofu when both gum Arabic and sodium alginate were zero. The coefficient for gum Arabic (-10.15) indicated that increasing the concentration of gum Arabic decreased tofu gumminess, while the coefficient of sodium alginate (-27.94) suggested that higher levels of sodium alginate significantly reduced tofu gumminess. This equation implied that adjusting the levels of gum Arabic and sodium alginate could effectively control the gumminess of tofu, with higher concentrations of these hydrocolloids generally resulting in a firmer, less gummy texture by modifying water retention and gel formation properties in tofu production. Zhou & Zheng (2017) highlighted that gum Arabic, known for its ability to stabilise and thicken, can reduce tofu gumminess by enhancing water binding and forming a cohesive gel network within the tofu matrix. This effect is noticeable at higher concentrations of gum Arabic, where tofu can become overly chewy or sticky. Similarly, sodium alginate, valued for its gel-forming properties, has been found to decrease gumminess by improving water retention and creating a softer texture in tofu.

According to Funami (2011), combining these hydrocolloids can have synergistic effects, further reducing gumminess compared to their individual use. Moreover, the interaction between hydrocolloids and other tofu ingredients, such as coagulants and soy protein, influences gumminess by altering the structural integrity and moisture content of tofu. In the study, increasing concentrations of gum Arabic tended to increase the gumminess of tofu. For instance, at a fixed sodium alginate level (e.g., 0.3% or 0.4%), increasing gum Arabic from 0.3% to 0.5% consistently resulted in higher gumminess values. This indicated that gum Arabic enhanced tofu's ability to form a cohesive and resilient gel structure, thereby increasing its chewiness. Similarly, higher concentrations of sodium alginate also contributed to increased gumminess of tofu. At a fixed gum Arabic level (e.g., 0.3% or 0.4%), increasing sodium alginate from 0.3% to 0.5% generally resulted in higher gumminess values, attributed to sodium alginate's water-binding and gel-forming properties that enhance tofu's firmness and chewiness. The interaction effect between gum Arabic and sodium alginate on gumminess was also evident. When both hydrocolloids were present in higher concentrations simultaneously (e.g., 0.5% gum Arabic and 0.5% sodium alginate), the gumminess in tofu tended to be significantly higher compared to when either hydrocolloid was used alone or at lower concentrations. This synergy suggested that their combined presence enhanced gel-forming properties, leading to a firmer texture in the tofu samples studied.

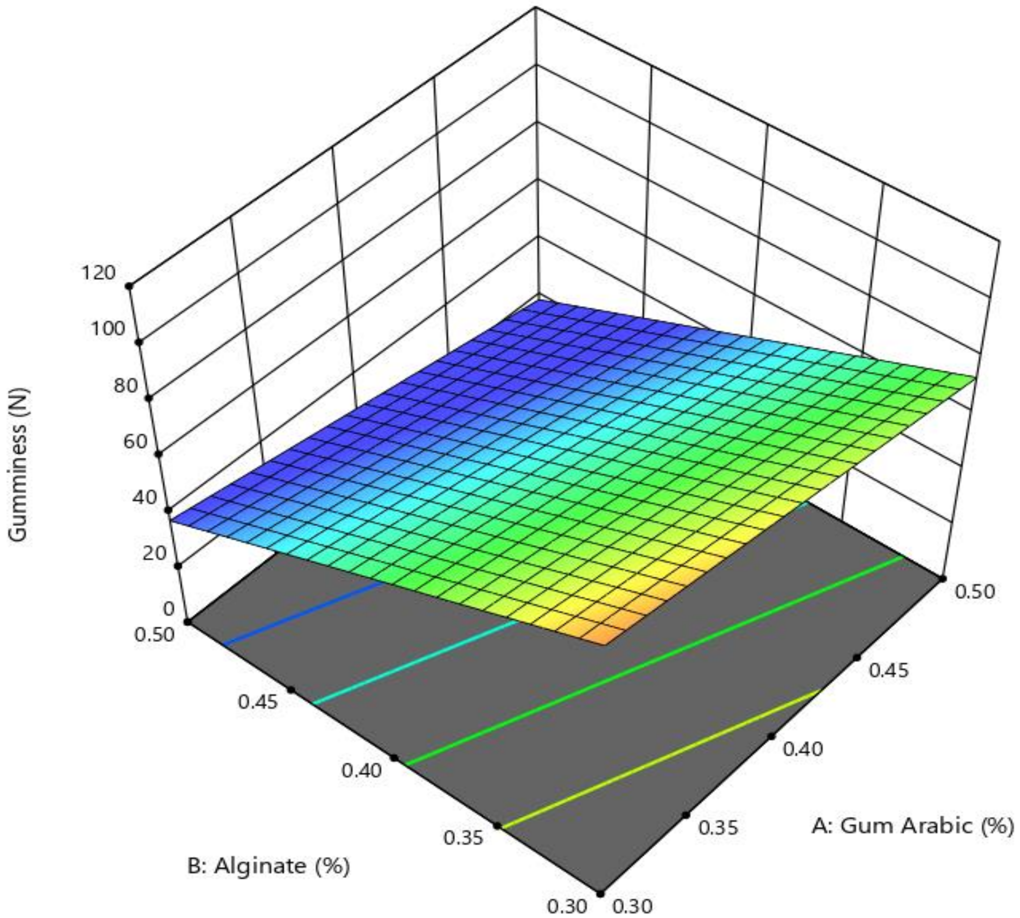


Figure 3.3: Effect of gum Arabic and sodium alginate on Bambara groundnut tofu gumminess.

The effect of gum Arabic and sodium alginate concentrations on BGN tofu colour properties is presented in Table 3.6. The lightness of tofu ranged from 68.50 [Run 10 (0.3 gum Arabic:0.5 sodium alginate)] to 82.36 [Run 7 (0.3 gum Arabic:0.3 sodium alginate)], while a* ranged from 1.58 [Run 4 (0.5 gum Arabic:0.3 sodium alginate)] to 5.02 [Run 7 (0.3 gum Arabic:0.3 sodium alginate)] and b* ranged from 6.03 [Run 2 (0.4 gum Arabic:0.5 sodium alginate)] to 9.60 [Run 12 (0.5 gum Arabic:0.5 sodium alginate)]. Run 7 (0.3 gum Arabic:0.3 sodium alginate) produced the lightest and reddest tofu while Run 12 produced the yellowest tofu. Bambara groundnut tofu prepared with 0.5:0.5 gum Arabic:sodium alginate differed significantly in redness when compared with tofu made with 0.3:0.3 gum Arabic:sodium alginate. The combination of 0.5:0.5 gum Arabic:sodium alginate appeared more yellow than other combinations. Gum Arabic and sodium alginate significantly ($p < 0.05$) affected the lightness, redness and yellowness of BGN tofu.

Table 3.6 **Effect of gum Arabic and sodium alginate concentration on Bambara groundnut tofu colour[1]**

Hydrocolloid		Colour parameter		
Gum Arabic (%)	**Sodium alginate (%)**	**Lightness (L*)**	**Redness/Greenness (a*)**	**Yellowness/Blueness (b*)**
0.3	0.3	82.36 ± 0.20[a]	5.02 ± 0.73[a]	9.14 ± 0.91[a]
0.3	0.4	72.42 ± 0.31[b]	1.99 ± 0.76[b]	8.24 ± 1.16[b]
0.3	0.5	68.50 ± 0.27[c]	2.25 ± 0.30[b]	8.05 ± 0.20[c]
0.4	0.3	75.18 ± 0.16[d]	3.81 ± 0.16[c]	8.01 ± 0.69[c]
0.4	0.4	76.74 ± 5.97[d]	3.39 ± 1.29[d]	8.91 ± 1.62[a]
0.4	0.5	70.37 ± 0.18[d]	4.43 ± 1.07[d]	6.03 ± 0.09[a]
0.5	0.3	76.87 ± 0.33[e]	1.58 ± 0.26[b]	6.44 ± 0.25[d]
0.5	0.4	80.58 ± 0.40[f]	1.73 ± 0.68[b]	8.24 ± 0.69[b]
0.5	0.5	79.49 ± 0.14[g]	3.36 ± 1.04[e]	9.60 ± 1.31[a,b]

[1]Mean values of triplicate determinations ± standard deviation. Means within a column followed by the same superscripts are not significantly ($p > 0.05$) different.

A high-quality tofu should be a light yellow or white in colour (Kim *et al*., 2019). Several researchers reported the lightness of soybean tofu in the range 81-88 (Yang *et al*., 2020), 86-88 (Ginting *et al*., 2021) and 86-87 (Kim *et al*., 2019). The tofu in this study was relatively darker than the reported soybean tofu and this could be attributed to the soybean seed and seed coat being cream-white in colour while mixed whole BGN seeds (cream, red and brown) were used in the production of tofu in this study. The colours of BGN seed coats affected the colour of the resultant tofu. However, the lightness of BGN tofu was still desirable, especially for [Run 7 (0.3 gum Arabic:0.3 sodium alginate)] (L* = 82.36), [Run 5 (0.5 gum Arabic:0.4 sodium alginate) (L* = 80.58) and [Run 12 (0.5 gum Arabic:0.5 sodium alginate)] (L* = 79.49). Despite being relatively darker in colour, Run 12 (0.5 gum Arabic:0.5 sodium alginate) was considered the optimum because of its desirable hardness and springiness.

Twelve BGN tofu formulations made with varying concentrations of gum Arabic and sodium alginate were successfully produced. Based on the optimisation goal of maximum hardness and springiness and minimum gumminess, the most desirable (desirability = 1) tofu was made with the gum Arabic (0.5%) and sodium alginate (0.5%). This formulation produced tofu with maximum hardness (9.11 N), maximum springiness (11.53 mm), minimum gumminess (20.7 N) and desirable colour (L* = 79.49, a* = 3.60, b* = 9.60). The range of gumminess from previous studies for soybean tofu is relatively low (0.00432-0.00549 N) (Dang *et al*., 2023). In contrast, the tofu in this study exhibited very high gumminess. High gumminess may be due to the addition of certain carbohydrate-based additives (gum Arabic, guar gum and carrageenan) during tofu making. The polysaccharides in these additives interact with the soy protein polymers, affecting the gel structure and texture characteristics of the tofu (Dang *et al*., 2023). Despite this, it was concluded that the tofu made with 0.5% gum Arabic and 0.5% sodium alginate was the optimum and was therefore used for further studies.

3.3.4 Texture profile of Bambara groundnut tofu

The influence of hydrocolloids on the tofu texture was noted with different compression failures. Figure 3.4 shows compressed BGN tofu coagulated with GDL with and without hydrocolloids.

a **b** **c**

Figure 3.4: Bambara groundnut tofu before and after compression. (a) Tofu before compression; (b) Tofu with hydrocolloids after compression; (c) Tofu without hydrocolloids after compression.

It was observed that tofu with 0.5:05 gum Arabic:sodium alginate greatly withstood compression, thereby achieving maximum hardness and springiness. In contrast, tofu made without hydrocolloids was extremely fragile and broke apart easily (Figure 3.4c). Moisture content had a great influence on the force deformation characteristics of BGN tofu. Tofu without hydrocolloids had a higher moisture content because of the absence of hydrocolloids, which aid in retaining water within tofu gel and thereby reduce the overall moisture content. Hydrocolloids like carrageenan, guar gum, and gum Arabic interact with soy proteins to form a more compact and stable gel network (Yuan & Chang, 2007).

The optimum proportion of gum Arabic and sodium alginate (0.5:0.5%) was significant in maintaining the structure of tofu after compression. Tofu with hydrocolloids retained its original condition after the deforming force was removed (Figure 3.4b). This can be attributed to the binding effect of gum Arabic and sodium alginate with the moisture within the tofu. The interaction of gum Arabic and sodium alginate increased tofu springiness and hardness. Springiness depends on factors such as heat treatment, protein interaction, flexibility and the degree of protein unfolding (Yuan & Chang, 2007; Yang *et al.*, 2020; Guan *et al.*, 2021). The springiness of the tofu without hydrocolloids (Figure 3.4c) was low, meaning the tofu did not retain its original form after compression.

The types of compression failures in tofu can be impacted by factors such as concentration and type of coagulant, pressing force applied, as well as storage conditions. Understanding these distinct failure modes is crucial for enhancing the production process and improving the overall quality and texture of tofu. The compression failure in Figure 3.4b is termed brittle failure. Brittle failure occurs when the material breaks apart suddenly under compressive forces, without any significant plastic deformation (Hamann *et al.*, 2006). It is characterised by the formation of sharp cracks or fracture surfaces and is often observed in firmer tofu. Thus, firmer tofu, which has a higher protein content and a more compact gel structure, is more prone to brittle failure during compression (Hamann *et al.*, 2006).

Figure 3.4c depicts ductile failure. In contrast to brittle failure, ductile failure is less prone to catastrophic breakdown and is commonly observed in soft tofu, which has a lower protein content and a less compact gel structure (Guan *et al.*, 2021). Different types of compression failures are a result of various factors, such as inadequate stirring during coagulation. Stirring is crucial for successful coagulation, and transitioning from hand-stirring to mechanical stirring is necessary to ensure proper mixing (Cao *et al*., 2022).
Figure 3.5 shows the difference in curd size of BGN tofu. Bambara groundnut tofu coagulated with GDL in the presence of gum Arabic and sodium alginate formed large curds (Figure 3.5a).

a **b**

Figure 3.5: Influence of hydrocolloids on curd size formation in Bambara groundnut tofu. (a) Curds with hydrocolloids. (b) Curds without hydrocolloids.

In contrast, tofu coagulated in the absence of gum Arabic and sodium alginate formed extremely fine curds (Figure 3.5b).

3.3.5 Expressible and entrapped water in Bambara groundnut tofu

The effect of coagulant (GDL and vinegar) and hydrocolloid (gum Arabic and sodium alginate) on the expressible and entrapped water of BGN tofu is presented in Table 3.7.

Table 3.7 Expressible and entrapped water of Bambara groundnut tofu[1]

Tofu	Expressible water (%)	Entrapped water (%)
0.3% GDL	20.93 ± 0.06[a]	56.07 ± 4.31[a]
0.6% GDL	19.27 ± 2.25[a]	56.47 ± 3.50[a]
1% GDL	27.27 ± 1.29[c]	45.13 ± 0.71[b]
4% Vinegar	21.07 ± 0.25[a]	56.33 ± 0.61[a]
0.3% GDL + 0.5% GA + 0.5% SA	17.70 ± 1.92[b]	62.80 ± 5.30[c]
0.6% GDL + 0.5% GA + 0.5% SA	17.13 ± 0.45[b]	65.60 ± 0.72[c]
1% GDL + 0.5% GA + 0.5% SA	24.83 ± 1.10[d]	50.00 ± 2.25[b]
4% Vinegar + 0.5% GA + 0.5% GA	21.17 ± 0.25[a]	55.20 ± 1.91[a]

[1]Values are mean ± standard deviation of triplicate analysis. Means within a column followed by the same superscripts are not significantly ($p > 0.05$) different. [2]GA: Gum Arabic; SA: Sodium alginate. GDL: Gluconolactone.

The moisture that can be removed from tofu by centrifugation is expressible water, while the remaining water is called entrapped water. High water content in tofu can induce syneresis, a process where water is expelled from the tofu matrix due to continuous rearrangements of protein molecules, leading to stress in the network and the breakage of protein bonds (Guan *et al*., 2021). The expressible water content of BGN tofu ranged from 17.13% (0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate) to 27.27% (1% GDL-tofu) and the entrapped water in BGN tofu ranged from 45.13% (1% GDL-tofu) to 65.60% (0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate). All GDL-tofu formulations without hydrocolloids differed significantly ($p < 0.05$) in the amount of expressible water compared to those with hydrocolloids. This was in agreement with the compression results (Section 3.3.4), where the addition of hydrocolloids reduced the water content of tofu by binding the water, hence increasing the hardness of the tofu. The 0.3% GDL-tofu and 0.6% GDL-tofu without hydrocolloids differed significantly ($p < 0.05$) in the amount of entrapped water from their counterparts with hydrocolloids. The addition of hydrocolloids to vinegar-tofu had no significant effect on the expressible and entrapped water content. The tofu with the highest expressible water (27.27%) was 1% GDL-tofu. These findings align with those of Inggrid & Hananjaya (2020), who reported that tofu with high GDL concentrations has a high water content. Gum Arabic and sodium alginate reduced the expressible water in tofu and could therefore significantly improve the quality, shelf life and overall acceptability of BGN tofu.

3.3.6 Proximate composition of Bambara groundnut tofu

Table 3.8 shows the proximate profile of BGN tofu. The moisture, ash, protein, fat and carbohydrate content of BGN tofu ranged from 65.52% (GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate) to 70.33% (0.6% GDL-tofu), 2.35% (vinegar-tofu + 0.5% gum Arabic + 0.5% sodium alginate) to 2.67% (0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate), 12.01% (0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate) to 14.24% (vinegar-tofu + 0.5% gum Arabic + 0.5% sodium alginate), 2.59% (0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate) to 9.02% (0.6% GDL-tofu) and 4.88% (0.6% GDL-tofu) to 17.21% (0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate), respectively. The addition of gum Arabic and sodium alginate significantly ($p < 0.05$) improved the proximate composition of BGN tofu. The effect of hydrocolloids was significantly ($p < 0.05$) different for ash, protein, fat and carbohydrates but did not differ significantly for the moisture content of BGN tofu. These findings suggest that these hydrocolloids can be used to enhance the nutritional profile and textural properties of BGN tofu. The moisture content in this study and that reported by Kim *et al.* (2019) showed a similar trend, with variations in moisture content (69.8% to 75.6%) based on the composition of the tofu samples. The moisture content of 0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate was 65.52%, while that of 0.6% GDL-tofu was significantly ($p < 0.05$) higher (70.33%).

This may be attributed to the absence of gum Arabic and sodium alginate in 0.6% GDL-tofu, which form a solid gel network structure. Gum Arabic and sodium alginate interact with the proteins in tofu and form a more compact and stable gel network that is able to retain water therefore resulting in a lower moisture content (Dey, 2017). Similar results were reported by Arii *et al*. (2021), where the moisture content of GDL-tofu was higher than that of calcium-sulphate and calcium acetate-tofu. The moisture content of Bambara groundnut tofu was lower than that of carrageenan-incorporated tofu (84%) and tamarind-coagulated tofu (71%) (Dey, 2017). Moreover, the vast network structure that hydrocolloids create has the potential to decrease syneresis by trapping water in the gel's interstitial spaces. The moisture content obtained from the 0.6% GDL, 0.5% gum Arabic, and 0.5% sodium alginate formulation aligns with the lower expressible water content reported in Section 3.3.5. The moisture content of tofu plays a critical role in determining its shelf life by influencing microbial growth, enzymatic activity, water activity, and texture changes. High moisture content creates a conducive environment for microbial proliferation, including bacteria, moulds, and yeasts (Dey, 2017). These microorganisms can cause spoilage, off-flavours, and textural changes in tofu, reducing its shelf life. Excessive moisture content can also lead to textural changes in tofu, making it softer and more prone to physical damage and spoilage (Preis, 2023).

Table 3.8 Proximate composition for Bambara groundnut tofu within coagulant[1]

Tofu	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
0.6% GDL	70.33 ± 2.84[a]	2.49 ± 0.03[a]	13.28 ± 0.10[a,b]	9.02 ± 0.34[a]	4.88 ± 3.08[a]
0.6% GDL + 0.5% GA + 0.5% SA	65.52 ± 1.87[b]	2.67 ± 0.01[b]	12.01 ± 1.11[a]	2.59 ± 1.31[b]	17.21 ± 1.70[b]
4% Vinegar + 0.5% GA + 0.5% SA	67.04 ± 0.61[a,b]	2.35 ± 0.07[c]	14.24 ± 0.23[b]	6.70 ± 2.21[a]	11.88 ± 4.84[b]

[1]Values are mean ± standard deviation of triplicate analysis. Means within a column followed by the same superscripts are not significantly ($p > 0.05$) different. [2]GA: Gum Arabic; SA: Sodium Alginate.

Furthermore, changes in texture can impact consumer acceptability and contribute to a shorter shelf life. The moisture content of BGN tofu from this study is lower than compared to the reports by Kim *et al*. (2019) and Preis, (2023), which were 76% and 81%, respectively. The moisture content of 0.6% GDL-tofu (70.33%) was similar to that reported by Dey (2017), which was 71.02%. Additionally, Dey (2017) reported that a lower soymilk solids content and longer coagulation time resulted in tofu with a higher moisture content (75-78%).

Vinegar-tofu had the highest protein and fat content and the lowest ash content. Gum Arabic and sodium alginate significantly ($p < 0.05$) affected the ash content of BGN tofu. Gluconolactone-tofu and vinegar-tofu significantly ($p < 0.05$) differed when compared with each other based on the ash content. This difference could be attributed to the different concentrations and types of coagulants used to make the tofu. Ash content is an indicator of the mineral content of food. The lowest ash content (2.35%) was obtained from 4% vinegar-tofu + 0.5% gum Arabic + 0.5% sodium alginate. The ash content showed slight variation, with the highest value (2.67%) in tofu prepared with 0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate. The tofu prepared with 4% vinegar + 0.5% gum Arabic + 0.5% sodium alginate tofu, with a protein content of 14.24%, qualified as a source of protein. According to the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972), a food product can be labelled as a source of protein if it contains a minimum protein content of 12% of the energy value (Anon., 2023). High protein content in BGN tofu makes it a nutritionally significant food item, especially for individuals following plant-based diets. The protein content of BGN tofu (with or without hydrocolloids) ranged from 12.01% to 14.24%, while soybean tofu is known for its high protein content (15.40%) as reported by to Dzikunoo *et al*. (2015). Sprouting soybeans can further enhance their protein content and digestibility.

The highest carbohydrate content (17.21%) and ash content (2.67%) were found in the 0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate. The addition of gum Arabic and sodium alginate increased the protein, carbohydrate and ash content of BGN tofu. This might be due to the proximate composition of these hydrocolloids. Gum Arabic and sodium alginate are long-chain polymeric carbohydrates connected by glycosidic bonds, which may contribute to the increased carbohydrate content in the tofu. Yebeyen *et al.* (2009) and Erben *et al.* (2019) reported that gum Arabic contains 1-3% protein, which could contribute to the increased protein content when incorporated into food products. Conversely, the lower protein content in the 0.6% GDL-tofu with 0.5% gum Arabic and 0.5% sodium alginate could be attributed to the GDL coagulant. GDL acidifies the soymilk gradually, curdling the soy proteins (USDA, 2016), but lower concentrations of GDL might not provide sufficient acidification for effective protein coagulation, potentially leading to lower protein retention. This acidification process leads to the curdling of the protein, causing it to separate from the liquid portion of the soymilk. Therefore, a lower concentration of GDL may not provide enough acidification to effectively coagulate and retain the protein content in the tofu, leading to a reduction in the protein content of the final tofu product. The 0.6% GDL-tofu had the highest moisture and fat content, and the lowest carbohydrate content. In contrast, the tofu made with 0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate had the highest ash and carbohydrate content, and the lowest protein and fat content. The lower protein and fat content in the 0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate compared to the 0.6% GDL-tofu without hydrocolloids suggests that the added hydrocolloids have negatively influenced protein and fat retention. This could be due to the altered pH conditions or the physical interactions between the coagulants and the BGN milk proteins and fats during coagulation. Moreover, raw materials such as salts influence the proximate composition of tofu.

The 0.6% GDL-tofu had the highest fat content (9.02%), which was not significantly different from vinegar-tofu (6.70%) but was significantly ($p < 0.05$) higher than 0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate. Bambara groundnut tofu and soybean tofu have similarities in moisture content, protein, fat, and carbohydrate contents. However, differences in coagulants and processing methods can affect the nutritional profile of tofu. The proximate values of BGN tofu obtained from this study were in agreement with previously reported data on tofu from other plant-based protein sources (Ndatsu & Olekam, 2012; Dey, 2017).

Tofu, typically derived from soybeans, is popular in Western countries. Its affordability, nutritional benefits, digestibility and versatile texture make it a dietary staple for many households, particularly those with lower incomes (Liu *et al*., 2004). It is prepared in various ways, such as frying, grilling, baking and incorporating into soups and stews. Figure 3.6 shows fried BGN tofu.

Figure 3.6: Fried Bambara groundnut tofu with mixed vegetables.

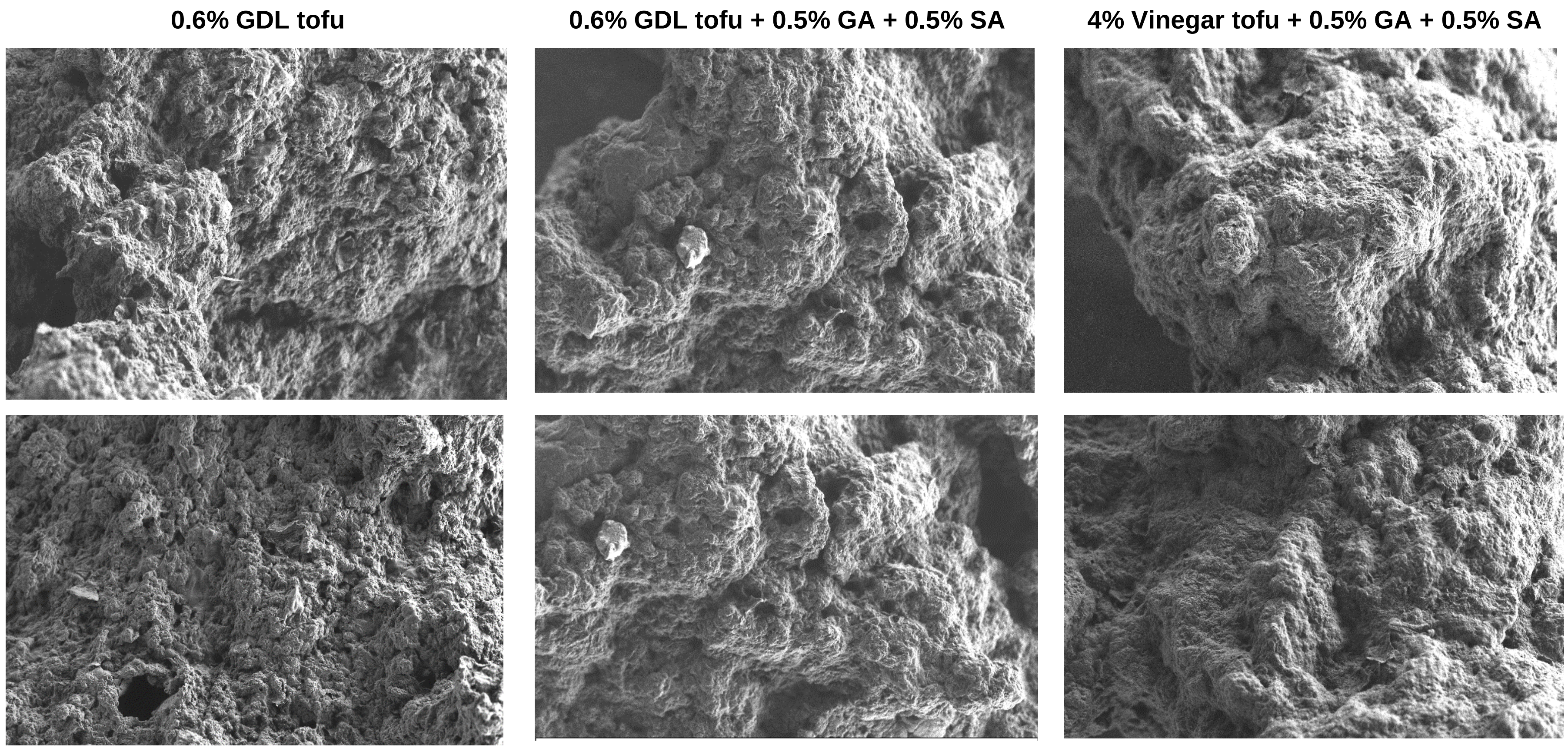
3.3.7 Microstructure of Bambara groundnut tofu

Scanning electron micrographs of BGN tofu at a resolution of 40 µm are shown in Figure 3.7. Scanning electron microscopy allows for the visualisation of the arrangement and characteristics of the protein and non-protein components in tofu, which is useful for studying the physicochemical structure of tofu (Dang *et al.*, 2023). The black areas in the SEM images represent the pores of the gel network (Shi *et al.*, 2020). The 0.6% GDL-tofu and 0.6% GDL-tofu + 0.5% gum Arabic + 0.5% sodium alginate exhibited larger particle sizes, coarse texture and large holes (Figure 3.7). Vinegar-tofu exhibited a more uniform, smaller particle size network with a smooth surface and small sized-holes. Larger holes are generally associated with high moisture tofu. For instance, the 0.6% GDL-tofu, which had higher moisture, exhibited more significant pore sizes (Table 3.8). The tofu components in GDL-coagulated tofu were more densely packed and formed distinct clusters. The uneven surfaces and size irregularities could be influenced by the type and concentration of coagulants used, as well as the incorporation of hydrocolloids or enzymes (Yasir *et al*., 2007; Shi *et al.*, 2020; Fan *et al.*, 2021; Hsieh *et al.*, 2022).

3.3.8 Effect of hydrocolloids on the rheological properties of Bambara groundnut milk extract

Figure 3.8 shows the flow behaviour of BGN milk extract prepared with 4% vinegar + 0.5% gum Arabic + 0.5 % sodium alginate (Figure 3.8a) and 0.6% GDL + 0.5 % gum Arabic + 0.5% sodium alginate (Figure 3.8b). The curve in Figure 3.8a exhibited a linear flow behaviour, while the curve in Figure 3.8b displayed a steep increase, indicating higher viscosity. This indicated that BGN milk prepared with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate was more viscous than that prepared with 4% vinegar + 0.5% gum Arabic + 0.5% sodium alginate. The increase in viscosity of BGN milk is attributed to electrostatic interactions between gum Arabic and sodium alginate with proteins. Complex formation between BGN proteins and polysaccharides occurs at a pH below the isoelectric point of the protein, where proteins are least soluble, and under low ionic strength conditions. The net charge of gum Arabic and sodium alginate decreases with the gradual attachment of protein macro-ion during the formation of electrostatic complexes (Ye, 2008).

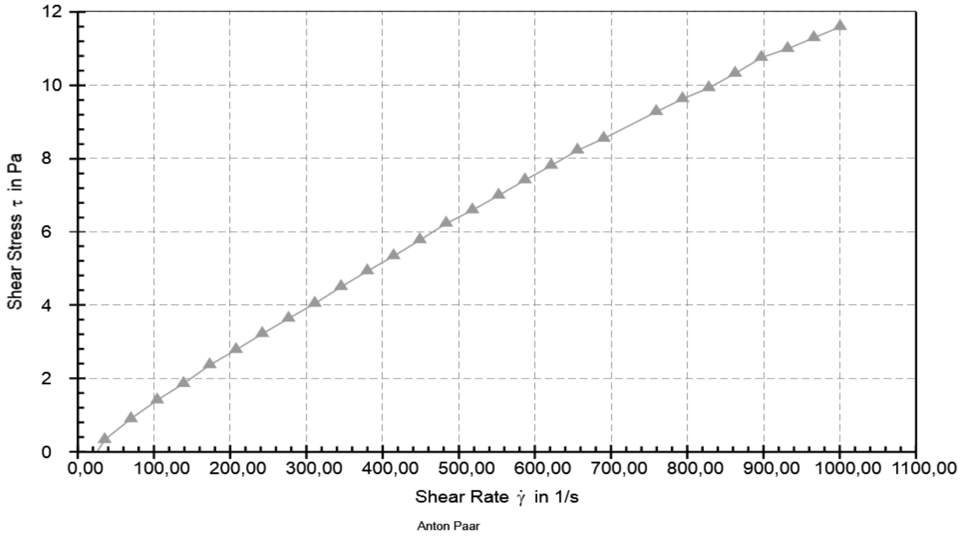
The rheological data of BGN milk extract was fitted into the Power law model. The Power law model is used in fluid characterisation and consists of two parameters, K and n (Pang *et al.*, 2021; Yuan *et al.*, 2022). The consistency coefficient (K) measures a system's viscosity, with lower values indicating a less viscous solution. The flow behaviour index (n) is a measure of a fluid's resistance to flow, with $n < 1$ indicating shear thinning and $n \geq 1$ predicting shear thickening (Pang *et al.*, 2021; Yuan *et al.*, 2022). Table 3.8 shows the Power law model parameters for BGN milk extract prepared with and without hydrocolloids.



a b c

Figure 3.7: Microstructure of Bambara groundnut tofu chunks (a) 0.6% GDL tofu (b) 0.6% GDL tofu + 0.5% gum Arabic + 0.5% sodium alginate (c) 4% Vinegar tofu + 0.5% gum Arabic + 0.5% sodium alginate.

a



b

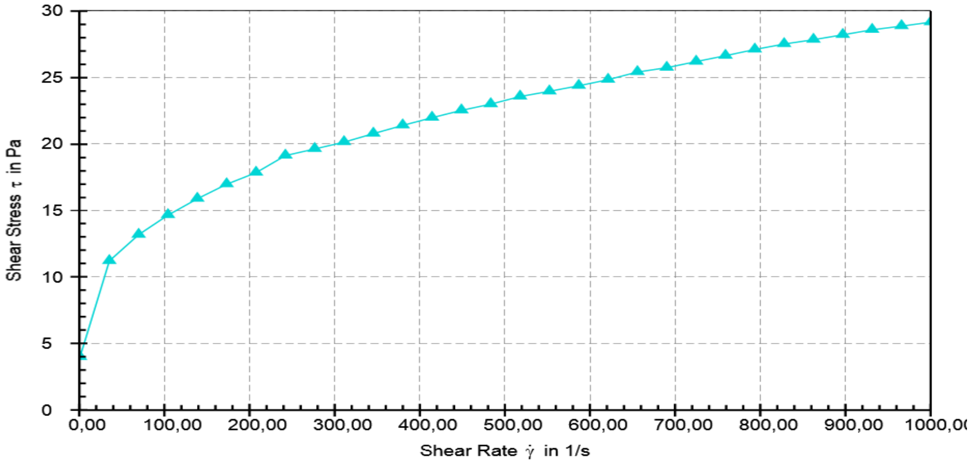


Figure 3.8: **Steady shear rheological parameters of Bambara groundnut milk extract with vinegar + 0.5% gum Arabic + 0.5% sodium alginate (a) and (b) 0.6% GDL +0.5% gum Arabic + 0.5% sodium alginate.**

Table 3.9 Power law model parameters for Bambara groundnut milk extract as affected by hydrocolloid type[1]

Tofu	K ($Pa.s^n$)	n	R^2
4% Vinegar	0.0215 ± 0.00^a	0.9085 ± 0.02^a	0.9781
0.3% GDL	0.0001 ± 0.00^b	1.4773 ± 0.02^b	0.9773
0.6% GDL	0.0001 ± 0.00^b	1.5083 ± 0.02^c	0.9772
1% GDL	0.0008 ± 0.00^c	$1.4964 \pm 0.02^{b,c}$	0.9772
4% Vinegar + 0.5% GA + 0.5% SA	0.0232 ± 0.00^d	0.9021 ± 0.00^a	0.9925
0.3% GDL + 0.5% GA + 0.5% SA	1.1095 ± 0.00^e	0.4065 ± 0.00^d	0.9148
0.6% GDL + 0.5% GA + 0.5% SA	1.5201 ± 0.00^f	0.4377 ± 0.00^e	0.9266
1% GDL + 0.5% GA + 0.5% SA	1.6850 ± 0.00^g	0.3816 ± 0.00^f	0.9025

[1]Values are mean ± standard deviation of triplicate analysis. Means within a column followed by the same superscript are not significantly ($p > 0.05$) different. [2]GA: Gum Arabic; SA: Sodium alginate. GDL: gluconolactone; K: consistency coefficient; n: flow behaviour index; R^2: coefficient of determination.

The Power law model was deemed suitable for describing the flow behaviour of BGN milk extract because the coefficient of determination (R^2) values were close to 1. The R^2 values were in the range 0.9025 (1% GDL + 0.5% gum Arabic + 0.5% sodium alginate) to 0.9925 (vinegar + 0.5% gum Arabic + 0.5% sodium alginate). The linear model was significant ($p < 0.05$) for both BGN milk extract with and without hydrocolloids. The effect of coagulant type on the rheological parameters (K, n and R^2) of BGN milk extract with and without gum Arabic and sodium alginate was significant ($p < 0.05$). The consistency coefficient (K) ranged from 0.0001 (0.3% GDL and 0.6% GDL) to 1.6850 $Pa.s^n$ (1% GDL + 0.5% gum Arabic + 0.5% sodium alginate). The lowest consistency coefficient (K) values were obtained from BGN milk extract without hydrocolloids. Although the consistency coefficient of BGN milk extract prepared with vinegar was greater than that of GDL formulations without hydrocolloids, it still exhibited non-Newtonian behaviour as its flow behaviour index (n) was less than 1. All milk extracts prepared with GDL and hydrocolloids exhibited higher viscosity ($n > 1$). Furthermore, the thickness of the systems increased with increasing GDL concentration. This concurred with Erben *et al*. (2019), who reported that a combination of gum Arabic and sodium alginate presents higher viscosity and elastic modulus.

The flow behaviour index (n) ranges from 0 for highly shear thinning materials to 1 for Newtonian materials. As such, the smaller the flow behaviour index (n) value, the greater the departure from Newtonian flow (Faustino & Pinheiro, 2021). The flow behaviour index (n) of BGN milk extract ranged from 0.3816 (1% GDL + gum Arabic + sodium alginate) to 1.5083 (0.6% GDL) (Table 3.8). All BGN milk extracts with gum Arabic and sodium alginate exhibited pseudoplastic (shear-thinning) behaviour ($n < 1$). All milk extracts prepared with GDL but without hydrocolloids exhibited shear thickening behaviour (n > 1), whereas those with GDL and hydrocolloids exhibited shear thinning behaviour (n < 1). Therefore, it can be deduced that the incorporation of hydrocolloids facilitated a transition from shear thickening to shear thinning behaviour in the systems.

The results of this study were in agreement with findings reported by Erben *et al*. (2019) who incorporated gum Arabic and sodium alginate to investigate their impact and interactions with whey protein isolate in the development of bio-based films. Figure 3.9 illustrates the flow behaviour of BGN milk extract with and without gum Arabic and sodium alginate. Gum Arabic and sodium alginate had a positive linear effect on the consistency coefficient of BGN milk extract coagulated with GDL (Figure 3.9). The consistency index was above 1 and the highest was exhibited by the milk extract prepared with 1% GDL + 0.5% gum Arabic + 0.5% sodium alginate. This was in agreement with the results shown in Table 3.8.

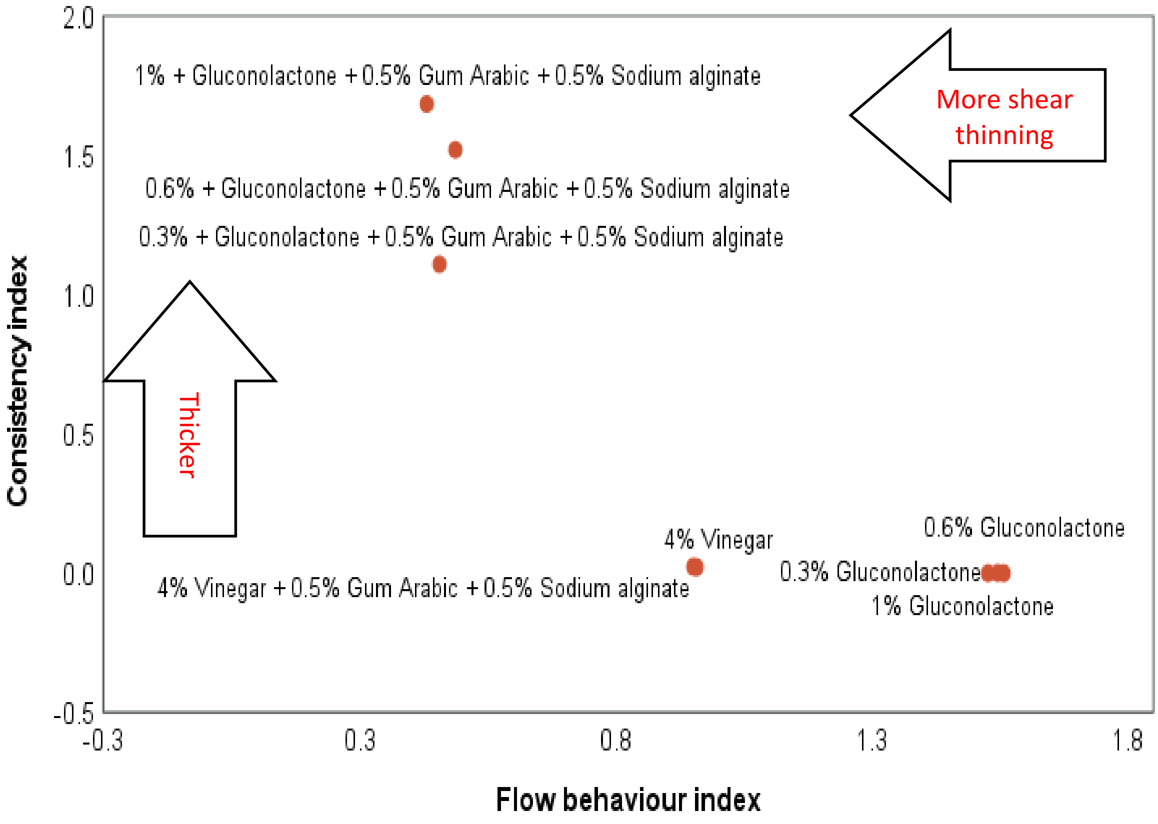


Figure 3.9: Flow behaviour of Bambara groundnut milk extract with and without gum Arabic and sodium alginate.

3.4 Conclusions

The effect of coagulants and hydrocolloids on the textural, physicochemical and rheological properties of BGN tofu was successfully investigated. The texture profile of BGN tofu was significantly affected by coagulants (GDL, vinegar and lemon juice) and hydrocolloids (gum Arabic and sodium alginate). An increase in coagulant concentration increased tofu hardness and decreased its springiness. Tofu coagulated with GDL and vinegar was lighter in colour, as well as firmer and springier in texture. The incorporation of gum Arabic and sodium alginate (05:05 w/w) produced tofu with a desirable texture profile: hardness (9.11 N) and springiness (11.53 mm). Gum Arabic and sodium alginate reduced the entrapped water in tofu, thereby suggesting that the resultant tofu would have a desirable texture, longer shelf life and meet consumer satisfaction. The network structure of vinegar-tofu was more uniform, smooth and exhibited fewer voids on the surface structure, implying a more compact and dense tofu. The 0.6% GDL-tofu with gum Arabic and sodium alginate had the lowest moisture and highest ash, while vinegar-tofu with gum Arabic and sodium alginate was a source of protein. The 0.6% GDL-tofu without gum Arabic and sodium alginate had the highest fat and lowest carbohydrate content. As such, the addition of gum Arabic and sodium alginate reduced the moisture and fat content of tofu while increasing the ash and carbohydrate content. Gum Arabic and sodium alginate can be successfully used to modify the textural, physicochemical and rheological characteristics of tofu and related products. Coagulant type and concentration, as well as the presence of hydrocolloids, significantly influenced the rheological behaviour of BGN tofu. Increasing GDL concentration on BGN milk extract prepared with gum Arabic and sodium alginate produced a viscous, shear-thickening tofu curds. The 1% GDL + 0.5% gum Arabic + 0.5% sodium alginate and 4% vinegar + 0.5% gum Arabic + 0.5% sodium gum Arabic were found desirable for enhancing great rheological properties of BGN tofu. Gluconolactone and vinegar, in combination with gum Arabic and sodium alginate, can be successfully used to modify the textural, physicochemical and rheological characteristics of tofu and related products.

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CHAPTER FOUR
FUNCTIONAL PROPERTIES AND CONSUMER ACCEPTABILITY OF BAMBARA GROUNDNUT TOFU CHUNKS

Abstract

Bambara groundnut (BGN) (*Vigna subterranea* [L.] Verdc.) chunks are a product from BGN tofu. Since BGN is a source of protein, it is suitable for the production of a healthy, high quality protein, meat-free product (BGN tofu chunks) to satisfy vegetarians, as well as promote personal health. This study established the functional and sensory qualities of BGN tofu chunks. Gum Arabic and sodium alginate had a significant ($p < 0.05$) positive effect on BGN tofu chunks. Incorporating gum Arabic and sodium alginate concentrations increased the lightness, redness and yellowness of BGN tofu chunks. The amino acid content of BGN tofu chunks revealed that it was among the food sources that can provide essential amino acids. Leucine (4.34-4.94%) and phenylalanine (4.61-5.62%) were the most abundant essential amino acids in all BGN tofu chunks samples. The content of lysine (3.76%) and alanine (2.65%) was higher for BGN tofu chunks made with 0.6% gluconolactone (GDL) compared to other samples. Bambara groundnut tofu chunks are a source of protein, cholesterol-free and have low moisture. Tofu chunks prepared with vinegar + 0.5% gum Arabic + 0.5% sodium alginate had the highest protein content (53.09%) while chunks prepared with 0.6% GDL had the lowest (42.70%). The protein content increased with the incorporation of gum Arabic and sodium alginate. Additionally, tofu chunks made with 0.6% GDL had a higher carbohydrate content ranging from 43.10 to 47.38%. The bulk density of BGN tofu chunks prepared with vinegar and 0.6 % GDL with 0.5% gum Arabic and 0.5% sodium alginate was similar. There was a significant increase in bulk density in tofu chunks prepared with 0.6% GDL as well as the ground chunk samples. Bambara groundnut tofu chunks with 0.6% GDL with 0.5% gum Arabic and 0.5% sodium alginate received the highest sensory scores for appearance, colour, taste, texture and overall acceptability compared to tofu chunks coagulated with vinegar and 0.6% GDL without gum Arabic and sodium alginate. Bambara groundnut tofu chunks possessed significant nutritional benefits and desirable physical properties, making them a potential substitute for animal protein products.

4.1 Introduction

Tofu, a traditional soybean-based food, is one of the most important soybean foods in Asian countries. Tofu has gained popularity worldwide due to its nutritional advantages and versatility (Xin *et al*., 2024). However, the exploration of alternative legume-based tofu products, such as Bambara groundnut (BGN) tofu, can contribute to the diversification of plant-based foods and ensure sustainability (Tan *et al*., 2020).

The formation of tofu is primarily attributed to the gelation property of soybean proteins (Qin *et al*., 2022). When soybean proteins are thermally denatured, heating induces the development of hydrophobic groups on the protein surface, leading to the formation of aggregates (Zeppa *et al*., 2021). When a coagulant is added, these aggregated proteins further interact to form polymerised proteins with a stable three-dimensional network structure (Xin *et al*., 2024). Soybeans contain 35-40% protein (dry weight), with the storage globulins 11S glycinin and 7S β-conglycinin accounting for 90% of this quantity (Singh *et al.*, 2015).

Tofu has nutritional advantages over other protein sources such as meat, fish, and cheese, including being cholesterol-free, rich in polyunsaturated fatty acids, and providing high-quality protein suitable for vegans as an alternative to animal-based foods (Qin *et al*., 2022; Xin *et al*., 2024). Legumes such as BGN are an excellent alternative to soybeans. Bambara groundnut is an indigenous African crop that offers a well-balanced diet with significant amounts of protein (18-24%), carbohydrates (57-63%), and fat (5-7%) (Ramatsetse *et al*., 2023). The vicilin (7S) protein fraction is the major storage protein fraction of BGN (Okpuzor & Okafor, 2010; Anjum *et al.*, 2011).

Properties of tofu chunks such as colour attributes, nutritional profile and sensory properties are important for consumer acceptability. Evaluating these properties is crucial for producing high-quality, meat-free protein sources that meet consumer preferences and health standards. Desirable BGN tofu chunks should possess a favourable amino acid profile, optimal texture, low bulk density, and low moisture content to ensure their marketability and consumer acceptability.

In Chapter 3, BGN tofu was successfully produced as a unique alternative to traditional soybean-based tofu. Dried tofu, also known as tofu chunks or plant meat, is a reprocessed soybean product made from tofu through drying, marinating, and sterilisation (Wu *et al*., 2023). Tofu chunks retain the nutritional composition of tofu, making them a balanced source of nutrients (Wu *et al*., 2023; Huang *et al*., 2022).

With increasing popularity of vegetarian and vegan diets, and the demand for sustainable food sources, tofu consumption is expected to rise in the coming years (Nadathur *et al.*, 2017; Gill *et al.*, 2024). Therefore, exploring the production of tofu chunks from BGN tofu can contribute to the diversification of products and ensure sustainability. However, the use of BGN for tofu and chunks production has not been previously reported. Since BGN chunks are a new product, sensory evaluation is important for understanding consumer perception, refining product characteristics, distinguishing between samples, and providing insights useful in determining target demographics. This study aimed to produce BGN tofu chunks and assess their functionality and consumer acceptability through sensory evaluation (including appearance, colour, taste, aroma, texture, and overall acceptability).

4.2 Materials and Methods

4.2.1 Source of materials and equipment

Bambara groundnuts were purchased from Triotrade, Johannesburg, South Africa. Gum Arabic, sodium alginate and gluconolactone (GDL) were purchased from Sigma-Aldrich (Pty) Ltd, South Africa. Food-grade vinegar and lemon juice were purchased from a local supermarket in Cape Town, South Africa. All other materials and equipment were obtained from the Department of Food Science and Technology of the Cape Peninsula University of Technology, Bellville Campus.

4.2.2 Bambara groundnut flour and tofu production

Bambara groundnut flour and BGN tofu used in this study were obtained according to the methods described in chapter 3, Sections 3.2.2 and 3.2.3, respectively.

4.2.3 Production of Bambara groundnut tofu chunks

Bambara groundnut chunks were produced from the three optimum tofu obtained according to Chapter 3 of this study (Section 3.2.6). The tofu was placed on a tray and firmly pressed using a plastic masher. A knife was then used to cut mashed tofu into evenly sized chunks. These chunks were dried in a dehydrator (Excalibur, Model No EXC10, NSF, USA) at 50ºC for 24 h and then analysed for textural and physicochemical properties, as well as consumer acceptability.

4.2.4 Proximate analysis of Bambara groundnut tofu chunks

The proximate composition of BGN tofu chunks was determined using the standard AOAC methods described in section 3.2.9

4.2.5 Amino acid profiling of Bambara groundnut tofu chunks

The amino acid composition of BGN tofu chunks was determined using the methods of Kowalska *et al.* (2022), Montevecchi *et al.* (2022) and Adewumi *et al.* (2022), with modifications. Milled fractions of BGN chunks (1 g) were placed into a 50 mL hydrolysis flask with 9 mL of 6 M hydrochloric acid and 2 mL of 10 mM internal standard (DL-2-aminobutyric acid). Additionally, 0.2 g of phenol was added to prevent the oxidation of certain amino acids.

Following this, flasks were sealed with Teflon and rubber septa, rendered inert with vacuum and ultra-pure nitrogen, and then heated to 110ºC for 24 h for the hydrolysis of proteins, yielding free amino acids. The hydrolysis flasks were taken out of the oven after 24 h and allowed to cool. The hydrolysate was filtered through filter paper. The filtrate (5 mL) was added to a 50 mL beaker and titrated with 12 M potassium hydroxide (KOH) to pH 9. The final volume was adjusted to 20 mL with distilled water. For derivatisation, the following reagents were sequentially added to 500 µL reaction tubes: 3 µL of sample, 237 µL of 100 mmol/L borate buffer (pH 9), 30 µL of 10 mmol/L potassium cyanide (pH 9), and 30 µL of 20 mmol/L NDA in acetonitrile. For 20 min, the derivatisation reaction was allowed to proceed at room temperature (24ºC). Following this, a fraction of the derivative solution was diluted five times with acetonitrile before injecting 25 µL into the high-performance liquid chromatography (HPLC) system. The column temperature was maintained at 40ºC. The mobile phases were acidified water with trifluoroacetic acid (TFA) at pH 2 (mobile phase A) and acetonitrile (mobile phase B). The solvent flow rate was set to 1.0 mL/min for 4.6 mm ID column. Measurements were carried out in triplicate.

4.2.6 Water absorption capacity of Bambara groundnut tofu chunks

The water absorption capacity (WAC) of BGN chunks were determined using the methods of Lorenzo *et al.* (2015), Gebrehiwot *et al.* (2018), Ezeama & Dobson (2019) and Samard *et al.* (2021). Tofu chunks (1 g) were weighed into a graduated centrifuge tube and then 10 mL of distilled water was added before vortexing thoroughly. The samples were allowed to stand for 30 min at room temperature (24ºC) and then centrifuged at 5ºC (Jouan, Centrifuge MR 18-12, Thermo Electron Corporation in France) at 5,000 x g for 30 min. The weight of the wet sample and the volume of free water were recorded. The WAC was calculated using Equation 4.1.

$$WAC\ (\%) = \left(W_2 - \frac{W_1}{W_1}\right) \times 100 \qquad (4.1)$$

Where WAC is the water absorption capacity (%), W_1 is the dry tofu chunks weight (g) and W_2 is the the wet tofu chunks weight (g).

4.2.7 Texture profile of Bambara groundnut tofu chunks

A texture analyser (Instron System ID Number 3344K6233, USA) was used to determine the compression strength of BGN chunks following the methods of Osen *et al.* (2014), Osen *et al*., (2015) & Samard & Ryu, (2019). Bambara groundnut tofu chunks (10 g) were transferred into an aluminium holding cup (Cat. No: S5403A), and a compression test consisting of first and second compression cycles was performed from 80 and 20% compression of the original sample height. The Kramer shear cell 5-bladed/KS5 (Cat. No: S5403A) cut through the chunks at a constant speed of 1.2 mm/s until fracture. The following parameters were quantified: the maximum force required to compress the sample (N), yield strength (MPa), compressive strength (MPa) and compressive strain (mm). Each measurement was carried out in triplicate and the results were recorded automatically on the Bluehills software.

4.2.8 Bulk density of Bambara groundnut tofu chunks

Bulk density measurement was carried out using the methods of George *et al.* (2021) and Adewumi *et al.* (2022). Tofu chunks (10 g) were transferred into a 10 mL measuring cylinder. The measuring cylinder was tapped steadily on a table surface until a constant volume was obtained. The measurements were carried out in triplicates, and the bulk density was calculated as the ratio of sample weight to the volume occupied by the sample (Equation 4.2).

$$Bulk\ density\ (g/mL) = \frac{Weight\ of\ sample\ (g)}{Volume\ of\ sample\ (mL)} \quad (4.2)$$

4.2.9 Microstructure of Bambara groundnut tofu chunks

The microstructure of BGN tofu chunks was determined using the scanning electron microscope (SEM) as described in Chapter 3, section 3.2.10.

4.2.10 Colour characteristics of Bambara groundnut tofu chunks

The colour properties of BGN tofu chunks were determined as described in Chapter 3, section 3.2.5.

4.2.11 Sensory analysis of Bambara groundnut tofu chunks

Three BGN tofu chunks prepared with (1) 0.6% GDL; (2) 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate and (3) 4% vinegar + 0.5% gum Arabic + 0.5% sodium alginate were cooked separately for sensory evaluation. Tofu chunks (1 kg) were soaked in hot water (95ºC) (1.5 L) for 30 min until soft. Excess water was drained from the chunks and kept aside. Onions (300 g) and green pepper (300 g) were sauteed in oil (20 mL) until golden. Thereafter, spices (5 g), mixed vegetables (200 g), tomatoes (150 g) and tofu chunks (990 g) were added and stired until the mixture was well cooked.

A consumer sensory analysis with 50 untrained panellists was conducted at the sensory evaluation laboratory in the Department of Food Science and Technology of Cape Peninsula University of Technology on the three cooked BGN chunks following the method of Loh & Breene (2007) and Bakhsh *et al.* (2021) with modifications. The panellists, consisting of students and staff, were each served warm cooked chunks (10 g) labelled with a three-digit random number. The samples were served in clear 40 mL tub containers with a small spoon and a serviette, all placed on black and white plastic trays. A cup of water was provided to rinse the palate before and between tasting. The panellists were asked to provide consent for participation in the study by signing the provided consent form before evaluating the samples. Each panellist received a score sheet with three coded samples and a 5-point hedonic scale, where 1 = dislike very much and 5 = like very much. The panellists were instructed to rate each sample individually based on its appearance, colour, taste, aroma, texture and overall acceptability.

4.2.12 Data analysis

The IBM Statistical Package for the Social Sciences (SPSS) version 27 (2020) was used for statistical analysis (Ezenwa & Iheme, 2022). All experiments were carried out in triplicate and results were expressed as mean ± standard deviation. Multivariate analysis of variance (MANOVA) was used to establish differences between treatments. Duncan's multiple range test was used to separate means where significant ($p \leq 0.05$) differences existed. Principal component analysis was used to identify patterns by reducing dimensionality in the data.

4.3 Results and Discussion

4.3.1 Proximate composition of Bambara groundnut chunks

Table 4.1 presents the proximate composition of BGN tofu chunks prepared with and without gum Arabic and sodium alginate. The moisture content ranged from 0.25 (0.6% GDL-tofu chunks + 0.5% gum Arabic + 0.5% sodium alginate) to 0.33% (0.6% GDL-tofu chunks); ash content from 0.40 (0.6% GDL-tofu chunks) to 0.65% (vinegar-tofu chunks + 0.5% gum Arabic + 0.5% sodium alginate); protein content from 42.70 (0.6% GDL-tofu chunks) to 53.09% (vinegar-tofu chunks + 0.5% gum Arabic + 0.5% sodium alginate); fat content from 7.02 (0.6% GDL-tofu chunks + 0.5% gum Arabic + 0.5% sodium alginate) to 15.21% (vinegar-tofu chunks + 0.5% gum Arabic + 0.5% sodium alginate) and carbohydrate content from 30.74 (vinegar-tofu chunks + 0.5% gum Arabic + 0.5% sodium alginate) to 47.38% (0.6% GDL-tofu chunks + 0.5% gum Arabic + 0.5% sodium alginate), respectively.

The moisture content of all BGN tofu chunks did not differ significantly. The BGN tofu chunks are a relatively low-moisture product. This low moisture content contributes to a longer shelf-life for the chunks making them less susceptible to microbial growth and spoilage, thereby allowing them to maintain their quality for a longer period compared to high-moisture tofu products.

Ash content in food indicates the amount of inorganic materials (minerals) remaining after organic matter is burned away (Shi *et al*., 2024). Chunks prepared with vinegar + 0.5% gum Arabic + 0.5% sodium alginate had the highest ash content (0.65%).

Table 4.1 Proximate composition for Bambara groundnut chunks as affected by coagulants and hydrocolloids[1]

	Proximate (%)				
Tofu chunks	**Moisture**	**Ash**	**Protein**	**Fat**	**Carbohydrate**
0.6% GDL	0.33 ± 0.12[a]	0.40 ± 0.09[a]	42.70 ± 1.06[a]	13.46 ± 2.14[a,b]	43.10 ± 1.89[a]
0.6% GDL + 0.5% GA +0.5% SA	0.25 ± 0.10[a]	0.50 ± 0.04[a]	44.88 ± 0.66[b]	7.02 ± 4.79[b]	47.38 ± 4.25[a]
4% Vinegar +0.5% GA +0.5% SA	0.32 ± 0.10[a]	0.65 ± 0.08[b]	53.09 ± 0.81[c]	15.21 ± 2.41[a]	30.74 ± 2.06[b]

[1]Mean ± standard deviation of triplicate determinations. Means within a column followed by different superscripts differ significantly ($p \leq 0.05$). [2]GA: Gum Arabic; SA: Sodium Alginate; GDL: Gluconolactone.

Compared to GDL, vinegar contributed more minerals to the tofu. Vinegar, especially that made from fermented sources, like apple cider, sugar cane or rice vinegar, can contain various minerals, including potassium, magnesium, and trace elements (Perumpuli & Dilrukshi, 2022; Antoniewicz *et al.*, 2022). Therefore, when used as a coagulant, these minerals may be introduced into the tofu and contribute to its overall mineral content.

Amongst the chunks, BGN tofu chunks made with vinegar, gum Arabic and sodium alginate had the highest protein content (53.09%), while tofu chunks made with 0.6% GDL had the least protein content (42.70%). All the chunks significantly ($p < 0.05$) differed from each other in terms of protein composition. The BGN tofu chunks had a high protein content, particularly when made with vinegar or gum Arabic and sodium alginate. Coagulants, such as vinegar, solidify proteins and fats in hot soymilk, thereby influencing the nutritional composition of tofu (Shi *et al*., 2024). Bambara groundnut tofu chunks are a high-quality protein source compared to soy-based textured vegetable protein (TVP).

The fat content of tofu chunks prepared with 0.6% GDL and 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate did not differ significantly. However, 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate differed significantly ($p < 0.05$) from BGN tofu chunks made with vinegar + 0.5% gum Arabic + 0.5% sodium alginate.

Tofu chunks made with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate had the highest (47.38%) carbohydrate content while those made with vinegar had the lowest (30.74%). The carbohydrate content of tofu chunks coagulated with GDL ranged from 43.10-47.38%. Tofu chunks made with 0.6% GDL and those made with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate did not differ significantly in carbohydrate content. However, both of these differed significantly ($p < 0.05$) from tofu chunks made with vinegar.

Bambara groundnut tofu chunks are a source of protein, naturally cholesterol-free, and have a low moisture content. The findings of this study are in agreement with the reports of Bakhsh *et al.* (2021) and Hong *et al.* (2022), which noted that soy-based TVP should contain 35-40% high-quality protein with a well-balanced amino acid profile, 15-20% fats and 30% carbohydrates. Additionally, other studies have reported that the incorporation of hydrocolloids such as gum Arabic and sodium alginate can increase the protein content and reduce the fat content of tofu, while also affecting other proximate properties depending on the specific coagulation method used. Ezeama & Dobson (2019) found that the use of different coagulants like GDL, calcium sulfate and magnesium chloride affected the proximate composition and isoflavone levels in tofu by increasing protein and fibre levels while decreasing fat and carbohydrate contents. Similarly, *Paz-Yépez et al*. (2024) found that the choice of coagulant influenced the nutritional value and *in vitro* protein digestibility of tofu. In this study, the choice of coagulant and the inclusion of hydrocolloids significantly affected the proximate composition of BGN tofu chunks, influencing their nutritional and functional properties.

4.3.2 Amino acid composition of Bambara groundnut chunks

Amino acids play a crucial role in both the nutritional benefits and sensory qualities of food. For instance, glutamic acid is a key component of umami, which enhances the flavour of food (Yamamoto & Inui-Yamamoto, 2023). Incorporating amino acids into the diet can help address issues such as excessive salt consumption, nutritional imbalances and deficiencies. Therefore, amino acid analysis can offer insights into a food's ability to meet an individual's amino acid requirements (Veldsman *et al*., 2023). Amino acids are categorised into two groups: essential and non-essential. Non-essential amino acids are produced or synthesised by human bodies while essential amino acids cannot be synthesised or produced by the body.

The amino acid composition of BGN tofu chunks is given in Table 4.2. In tofu chunks prepared with vinegar, gum Arabic, and sodium alginate, glutamic acid had the highest concentration (11.38 g/100 g) while glycine had the lowest (1.10 g/100 g). Glutamic acid is a non-essential amino acid important for metabolism and neurotransmission. Glycine is also a non-essential amino acid important for protein synthesis, collagen formation, and metabolic processes. Among the essential amino acids, leucine had the highest concentration for all tofu chunks ranging above 4 g/100 g, while methionine had the lowest concentration of 1 to 2 g/100 g. Tofu chunks prepared with vinegar + 0.5% gum Arabic + 0.5% sodium alginate had the highest concentration of methionine and phenylalanine. For tofu chunks prepared with 0.6% GDL without hydrocolloids, the non-essential amino acids ranged from 1.86 (Glycine) to 10.03 (Glutamic acid) g/100 g. In contrast, tofu chunks made with GDL combined with gum Arabic and sodium alginate had non-essential amino acid levels ranging from 1.77 (Glycine) to 9.82 (Glutamic acid) g/100 g. Meanwhile, tofu chunks prepared with vinegar + 0.5% gum Arabic + 0.5% sodium alginate showed a wider range, with non-essential amino acids varying from 1.10 (Glycine) to 11.38 (Glutamic acid) g/100 g. For essential amino acids, 0.6% GDL-tofu chunks ranged from 1.65 (Methionine) to 4.82 (Phenylalanine) g/100 g, while tofu chunks prepared with vinegar combined with gum Arabic and sodium alginate had the highest values ranging from 2.01 (Threonine) to 5.62 (Phenylalanine) g/100 g. Overall, methionine had the lowest concentration among the essential amino acids in all tofu chunks. The low methionine levels found in this study are consistent with those observed in other legumes, such as soybeans (1.40 g/100 g) and chickpeas (0.11 g/100 g) (Adebowale *et al.*, 2011; Veldsman *et al*., 2023). The high lysine and low methionine content reflect the typical amino acid composition of BGN and other legumes (Adebowale *et al*., 2011). Additionally, the use of additives such as gum Arabic and sodium alginate, as well as coagulants like GDL and vinegar, can influence the amino acid profile of tofu by either increasing or decreasing its composition.

Table 4.2 Amino acid (g/100 g) composition of Bambara groundnut chunks within coagulant[1]

Amino acid	0.6% Gluconolactone (g/100 g)	+0.5% Gum Arabic + 0.5% Sodium alginate	
		0.6% Gluconolactone (g/100 g)	4% Vinegar (g/100 g)
Arginine[1]	3.76 ± 0.06[a]	3.66 ± 0.01[a]	4.24 ± 0.03[b]
Serine[1]	3.13 ± 0.07[a]	2.91 ± 0.01[b]	3.37 ± 0.03[c]
Glycine[1]	1.86 ± 0.02[a]	1.77 ± 0.01[b]	1.10 ± 0.01[c]
Asparagine[1]	6.79 ± 0.04[a]	6.45 ± 0.08[b]	7.55 ± 0.11[c]
Glutamic acid[1]	10.03 ± 0.01[a]	9.82 ± 0.01[b]	11.38 ± 0.05[c]
Alanine[3]	2.65 ± 0.01[a]	2.61 ± 0.04[a]	2.63 ± 0.03[a]
Proline[3]	2.17 ± 0.01[a]	2.01 ± 0.01[b]	2.41 ± 0.04[c]
Threonine[2]	2.06 ± 0.01[a]	1.85 ± 0.01[b]	2.16 ± 0.01[c]
Histidine[2]	2.21 ± 0.07[a]	2.39 ± 0.01[b]	2.59 ± 0.02[c]
Lysine[2]	3.76 ± 0.02[a]	3.63 ± 0.11[a]	3.29 ± 0.11[b]
Tyrosine[2]	2.75 ± 0.05[a]	2.59 ± 0.04[a]	3.12 ± 0.13[b]
Methionine[2]	1.65 ± 0.06[a]	1.69 ± 0.04[a]	2.01 ± 0.08[b]
Valine[2]	2.65 ± 0.06[a]	2.54 ± 0.04[a]	2.86 ± 0.04[b]
Isoleucine[2]	2.27 ± 0.04[a]	2.17 ± 0.04[a]	2.45 ± 0.03[b]
Leucine[2]	4.53 ± 0.01[a]	4.34 ± 0.06[a]	4.94 ± 0.06[b]
Phenylalanine[3]	4.82 ± 0.12[a]	4.67 ± 0.18[a]	5.62 ± 0.13[b]

[1]Values are mean ± standard deviation of triplicate determinations. Means within a row followed by the different superscript are significantly ($p > 0.05$) different. [2]Essential amino acid [3]Non Essential amino acid.

Tofu chunks made with GDL and vinegar had relatively high levels of phenylalanine. Furthermore, both leucine and phenylalanine were the most abundant essential amino acids across all BGN tofu chunks. The content of non-essential amino acids also varied depending on the coagulants used. Among the non-essential amino acids, glycine had the lowest concentration, while glutamic acid had the highest concentration in all BGN tofu chunks.

The amino acid content presented in Table 4.2 indicates that BGN tofu chunks are rich in essential amino acids such as leucine, lysine and phenylalanine. These findings agree with the findings of several studies, which observed that aspartic and glutamic acids are the most abundant in legumes and nuts (Adebowale *et al*., 2011, Adeyeye *et al*., 2012 and Nyau *et al*., 2019). Furthermore, the levels of essential amino acids (isoleucine, lysine, phenylalanine, and methionine) in BGN tofu chunks were within the recommendations set by the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO) (Bandyopadhyay *et al*., 2022). When compared to the recommended dietary allowances (RDA), the levels of essential amino acids in BGN tofu chunks, such as isoleucine (2.27-2.45 g/100 g), lysine (2.90-3.76 g/100 g), methionine (1.65-2.01 g/100 g) and phenylalanine (4.67-5.62 g/100 g) meet or exceed the RDA values for adults. This suggests that BGN tofu chunks can effectively contribute to meeting dietary requirements for these essential amino acids.

The higher lysine content of BGN tofu chunks is a significant nutritional benefit, making them an excellent complementary protein source for cereals that are typically low in lysine. Additionally, arginine-rich proteins are known for their potential protective effects against heart disease, while glutamine supports the immune system. Pairing cereals with lysine-rich foods such as legumes helps achieve a more balanced and complete protein intake. Moreover, combining cereals and legumes not only addresses the amino acid deficiency but also enhances the overall nutrient density of the food, resulting in a more well-rounded nutritional profile.

Figure 4.1 illustrates the distribution of various tofu chunks along the principal components, explaining their variance in amino acid composition. Component 1 accounts for 78.9% of the variance, while component 2 accounts for 21.1% of the variance, indicating that component 1 is more significant in differentiating the samples. Component 1 positively correlates with the tofu made with 4% vinegar + 0.5% gum Arabic + 0.5% sodium alginate, which is high in arginine, glycine, tyrosine, methionine, isoleucine, leucine and phenyalanine, while also showing that 0.6% GDL high in alanine and average in serine and threonine. Component 2, which consists of 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate, is high in histidine content (2.39 g/100 g).

Overall, BGN tofu chunks are notably rich in essential amino acids, making them a valuable dietary addition for improving overall protein intake and nutritional balance. Therefore, incorporating foods high in essential amino acids into the diet can be a valuable strategy to tackle nutritional concerns.

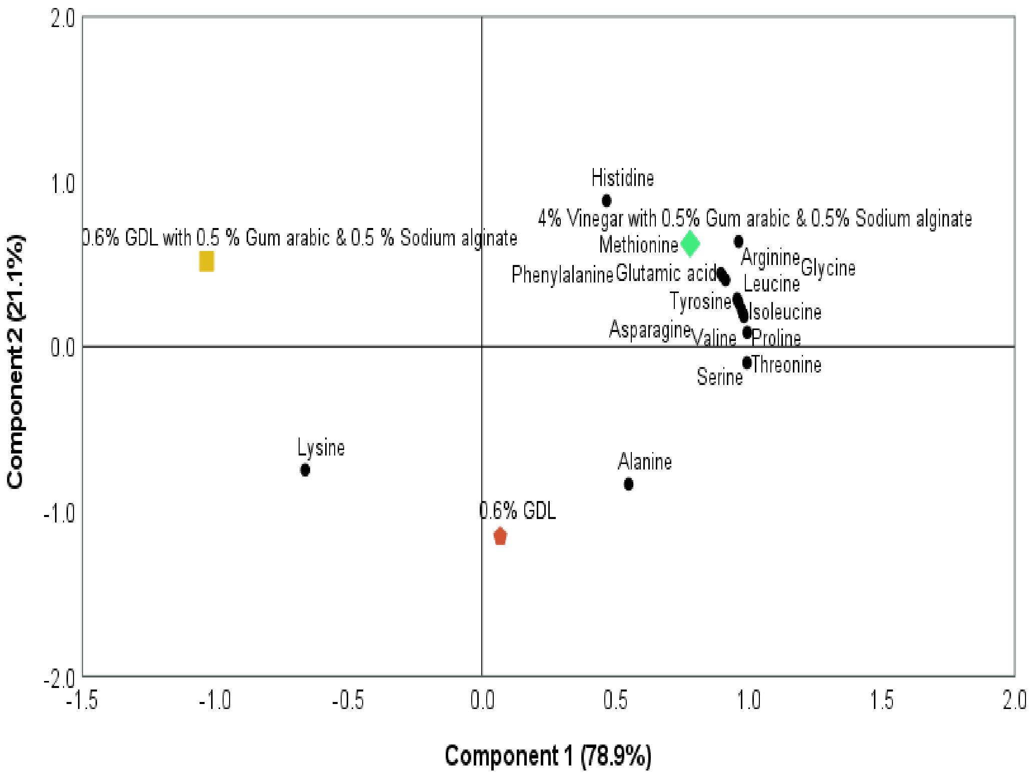


Figure 4.1: Principal component biplot of the amino acid profile of Bambara groundnut tofu chunks prepared with 0.6% GDL, 0.6% GDL + 0.5% GA + 0.5% SA and 4% vinegar + 0.5% GA + 0.5% SA.

4.3.3 Water absorption capacity of Bambara groundnut tofu chunks

Water absorption is the ability of a material to absorb water when immersed in it (Ezeama & Dobson, 2019). This property is crucial for retaining moisture in food. Soaking tofu chunks before cooking softens their structural proteins, making them easier to cook. Consequently, soaking BGN tofu chunks increases their water content, which accelerates the chemical reactions during cooking and improves the texture. Table 4.3 depicts the WAC of BGN tofu chunks, highlighting the effects of different coagulant types and the addition of hydrocolloids (gum Arabic and sodium alginate). The WAC of BGN tofu chunks ranged from 0.85 to 1.30 g/mL for tofu chunks prepared with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate and vinegar + 0.5% gum Arabic + 0.5% sodium alginate, respectively. There was a significant difference ($p \leq 0.05$) in the WAC of tofu chunks made with GDL and those made with vinegar. The results indicated that adding gum Arabic and sodium alginate with GDL slightly reduced water absorption, suggesting that these hydrocolloids may help stabilise the tofu matrix, thereby limiting its capacity to absorb water.

Table 4.3 Water absorption of Bambara groundnut tofu chunks soaked for 60 minutes[1]

Tofu chunks	Water absorption capacity (g/mL)
0.6% GDL	0.91 ± 0.24[a]
0.6% GDL + 0.5% GA + 0.5% SA	0.85 ± 0.18[a]
4% Vinegar + 0.5% GA + 0.5% SA	1.30 ± 0.32[b]

[1]Values are mean ± standard deviation of triplicate analysis. Means within a column followed by different superscripts are significantly ($p \leq 0.05$) different. [2]GA: Gum Arabic; SA: Sodium alginate. GDL: Gluconolactone.

Conversely, the inclusion of gum Arabic and sodium alginate, along with vinegar as a coagulant, seemed to enhance the WAC of the tofu chunks. Moreover, increased water absorption may be attributed to the properties of gum Arabic and sodium alginate, which are known for their ability to bind water and contribute to the texture and stability of food products (Taheri & Jafari, 2019).

Tofu chunks made with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate had a lower WAC compared to those made with 0.6% GDL without gum Arabic and sodium alginate. The difference in WAC can be attributed to different coagulants used during the making of tofu, which influences the structure of the protein and polysaccharide complexes formed (Li *et al*., 2019). The lower WAC in tofu made with gum Arabic and sodium alginate may result in a firmer, more compact texture compared to tofu made with GDL alone, which could influence the mouthfeel and overall sensory perception of the product. Tofu chunks with lower WAC may be suitable in recipes that require a firmer texture or in products that need to maintain their shape during processing or handling.

The effect of time on the water absorption of BGN tofu chunks is shown in Figure 4.2. Bambara groundnut tofu chunks prepared with vinegar + 0.5% gum Arabic + 0.52% sodium alginate exhibited maximum WAC of 1.70% at 40 min. Tofu chunks made with 0.6% GDL and 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate had lowest WAC compared with vinegar across all time intervals. Increasing the soaking time of tofu chunks increased the WAC. However, a decrease in WAC (to 1.49%) was noted at 50 min for tofu chunks made with vinegar + 0.5% gum arabic + 0.5% sodium alginate, although this value remained the highest among the chunks made with 0.6% GDL. The decrease in moisture intake could be attributed to the system reaching equilibrium and the release of other substances. Olson (2023) and Melvani (2023) highlighted that soaking tofu in salted water for 15 min draws out excess water from the tofu, resulting in a better crust and texture in the dried product. The removal of excess water through the pre-treatment step of soaking tofu chunks in salted water effectively enhanced the quality and characteristics of the dried tofu. The results from this study demonstrate that soaking chunks in water not only tenderises them but also enhances their ability to mimic the chewy texture of meat, allowing them to better absorb flavours from spices and sauces during cooking.

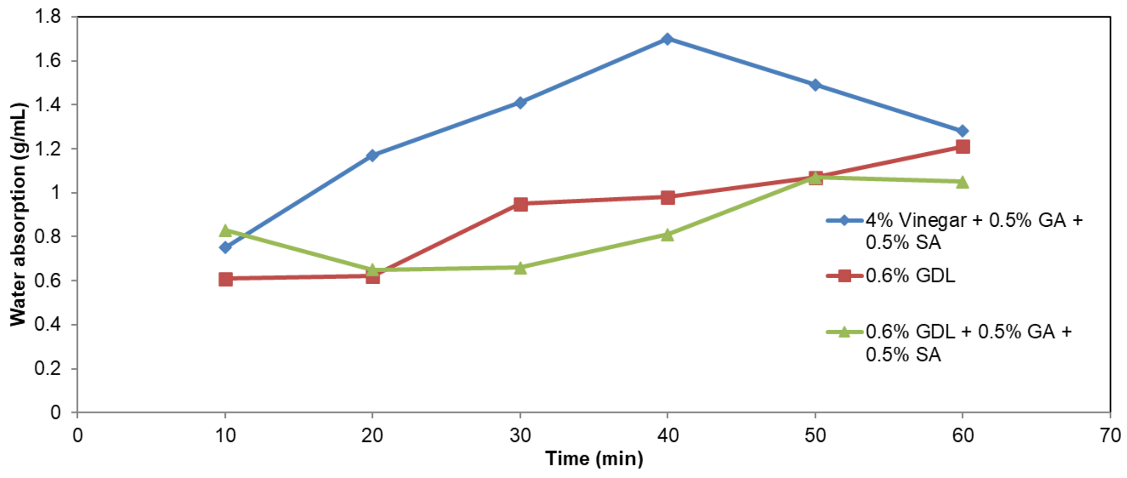


Figure 4.2: Effect of time on water absorption capacity of Bambara groundnut tofu chunks prepared with 0.6% GDL, 0.6% GDL + 0.5% GA + 0.5% SA and 4% vinegar + 0.5% GA + 0.5% SA.

4.3.4 Textural parameters of Bambara groundnut tofu chunks

The effect of coagulant type, gum Arabic, and sodium alginate on the compression parameters of BGN tofu chunks was investigated. The BGN tofu chunks did not differ significantly in compression parameters when compared to each other (Table 4.4).

Table 4.4 Effect of coagulant type and gum Arabic and sodium alginate on compression parameters of Bambara groundnut chunks[1]

Tofu chunks	Maximum compression (Kgf)	Yield strength (MPa)	Compression strain extension (mm)	Compressive strength (MPa)
0.6% GDL	80.97 ± 67.96[a]	0.01 ± 0.01[a]	0.32 ± 0.33[a]	0.32 ± 0.27[a]
0.6% GDL + 0.5% GA + 0.5% SA	42.78 ± 65.08[a]	0.13 ± 0.21[a]	0.45 ± 0.14[a]	0.17 ± 0.25[a]
4% Vinegar + 0.5% GA + 0.5% SA	42.87 ± 67.85[a]	0.01 ± 0.01[a]	0.60 ± 0.33[a]	0.17 ± 0.25[a]

[1]Values are mean ± standard deviation of triplicate analysis. Means within a column followed by different superscripts are significantly ($p \leq 0.05$) different. [2]GA: Gum Arabic; SA: Sodium Alginate. GDL: Gluconolactone.

The compression parameters of BGN tofu chunks ranged from 42.78 (0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate) to 80.97 Kgf (0.6% GDL) for maximum load, 0.01 (vinegar-tofu chunks and 0.6% GDL) to 0.13 MPa (0.6% GDL + 0.5% gum Arabic + 0.5%

sodium alginate) for yield strength, 0.32 (0.6% GDL) to 0.60 mm (vinegar-tofu chunks) for compressive strain extension and 0.17 (vinegar-tofu chunks and 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate) to 0.32 MPa (0.6% GDL) for compressive strength. Bambara groundnut tofu chunks made with only 0.6% GDL exhibited a greater force and a compressive strength than tofu chunks made with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate. The higher compressive strength suggests that BGN chunks may hold their shape better during cooking making them more versatile in various recipes. The chunks with hydrocolloids had a softer texture than those without hydrocolloids.

The choice of coagulant, gum Arabic, and sodium alginate did not significantly impact the compression parameters of BGN tofu chunks. According to Darmajana *et al*. (2020) this can be attributed to the inherent properties and composition of the BGN matrix, which may be the primary factor influencing the textural and structural characteristics of the tofu chunks, with the type of coagulant playing a secondary role. The dominance of the BGN matrix properties could overshadow any potential effects of the coagulant used (Darmajana *et al*., 2020). Specific compression parameters measured, such as maximum compression and yield strength, may not have been sensitive enough to detect subtle differences in texture and structure due to the coagulant type. Therefore, more advanced or specialised texture analysis methods may be required to elucidate the impact of coagulants on the BGN tofu characteristics. The limitations of the compression testing method used in this study may have hindered the ability to capture the nuanced effects of the coagulant type on the final texture and structure of the BGN tofu chunks.

4.3.5 Bulk density of Bambara groundnut tofu chunks

Bulk density is a measure of how much mass is contained within a given volume (Pugliese *et al*., 2017). The bulk density of BGN tofu chunks ranged from 0.29 (vinegar-tofu chunks) to 0.37 g/mL (0.6% GDL-tofu chunks) (Table 4.5).

Table 4.5 The bulk density of tofu chunks[1]

Tofu chunks	**Bulk density (g/mL)**
0.6% GDL	0.37 ± 0.01[a]
0.6% GDL + 0.5% GA + 0.5% SA	0.32 ± 0.03[b]
4% Vinegar + 0.5% GA +0.5% SA	0.29 ± 0.01[b]
Chunks powder	
0.6% GDL	0.69 ± 0.02[a]
0.6% GDL + 0.5% GA + 0.5% SA	0.65 ± 0.01[b]
4% Vinegar + 0.5% GA + 0.5% SA	0.73 ± 0.01[c]

[1]Values are mean ± standard deviation of triplicate analysis. Means within a column followed by different superscripts are significantly ($p \leq 0.05$) different. [2]GA: Gum Arabic; SA: Sodium alginate. GDL: Gluconolactone.

This implied that the choice of coagulant affected the density of the tofu, with GDL resulting in denser tofu chunks than vinegar. Ground chunks had higher bulk density values than tofu chunks, ranging from 0.65 to 0.73 g/mL for 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate and vinegar 0.5% gum Arabic + 0.5% sodium alginate, respectively. Gum Arabic and sodium alginate had no significant effect on the bulk density of tofu chunks. However, both gum Arabic and sodium alginate reduced the bulk density of the chunks compared to 0.6% GDL-tofu chunks. This suggests that the incorporation of these additives had a densifying effect on the powdered tofu.

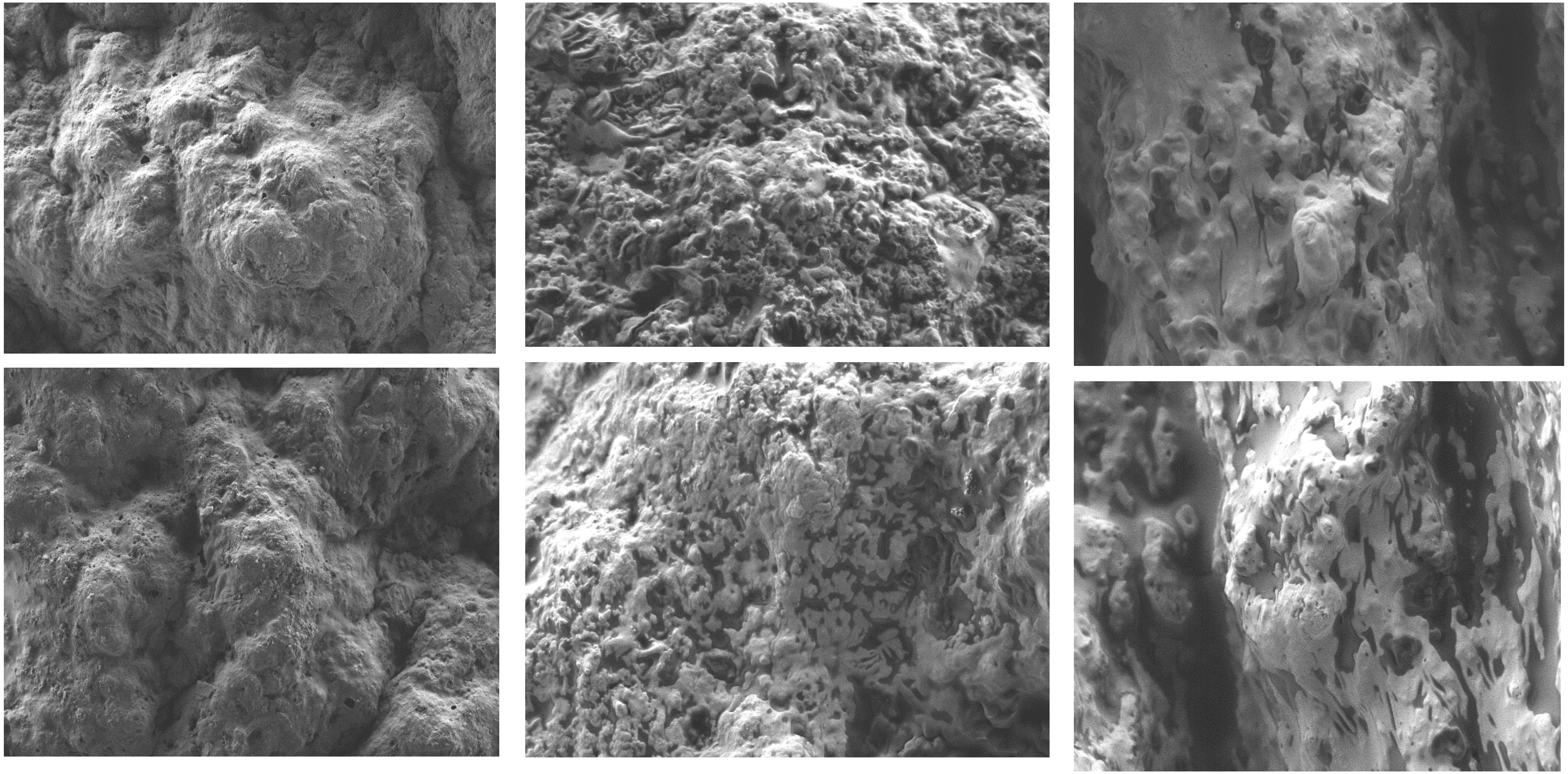
The use of GDL, gum Arabic, and sodium alginate can impact the density of tofu chunks, with GDL leading to denser tofu chunks (Ibrahim & Karim, 2024). The results provide valuable insights into the factors that influence the density and texture of tofu, which can be significant for its utilisation in food preparation and processing. Denser tofu chunks may occupy less volume, allowing for more efficient use of space in transporting containers and storage facilities.

4.3.6 Microstructure of Bambara groundnut tofu chunks

Scanning electron micrographs of BGN tofu chunks coagulated with 0.6% GDL, 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate and 4% vinegar + 0.5% gum Arabic + 0.5% sodium alginate at 20 µm resolution are shown in Figure 4.3. The network structure appeared different in all pictures, although the surface of Figures 4.3a and b exhibited a smooth texture. The microstructures depicted in the images correspond to the visually observed textures and are consistent with the results reported in Table 4.4. Tofu chunks coagulated with 0.6% GDL exhibited a uniform, smooth surface with small holes. These chunks were judged to have the best compression characteristics compared to those coagulated with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate and 4% vinegar + 0.5% gum Arabic + 0.5% sodium alginate. The chunks prepared with gum Arabic and sodium alginate displayed a clustered arrangement with round-shaped components. The micrographs also revealed two distinct layers, light grey and darker, which could be attributed to the hydrocolloid inclusions in Figures 4.3a and b. The absence of holes in these images suggests a more compact structure with higher protein components.

Furthermore, the network in Figure 4.3b appeared more continuous and finer, which was associated with lower water absorption (Table 4.4). Similar findings were reported by Zhang *et al*. (2023b), where GDL-coagulated tofu had the most uniform and compressible texture, while the inclusion of gum Arabic and sodium alginate resulted in a more layered and clustered microstructure that was harder, chewier and less water absorbent. This effect is likely due to the gel-strengthening properties of the hydrocolloids (Lee *et al*., 2024).

0.6% GDL | **0.6% GDL + 0.5% GA + 0.5% SA** | **4% Vinegar + 0.5% GA + 0.5% SA**



a | **b** | **c**

Figure 4.3: **Microstructure of Bambara groundnut tofu chunks prepared with (a) 0.6% GDL chunks (b) 0.6% GDL + 0.5% GA + 0.5% SA (c) 4% vinegar + 0.5% GA + 0.5% SA. All tofu chunks were examined at 20 µm resolution.**

Sodium alginate, in particular, can enhance gel strength by cross-linking with divalent cations, contributing to increased hardness in tofu and its chunks. However, the effect depends on the specific composition and arrangement of the alginate, with higher GDL content and block structures leading to stronger gels. The addition of hydrocolloids like gum Arabic and sodium alginate increases the hardness, chewiness and cohesiveness of starch-hydrocolloid complexes similar to tofu, compared to controls without hydrocolloids. Gum Arabic also provides excellent thickening and stabilising properties in food systems (Lee *et al*., 2024).

4.3.7 Bambara groundnut chunks colour characteristics

The colour characteristics of BGN tofu chunks, including lightness (L*), redness (a*) and yellowness (b*), are presented in Table 4.6. The lightness of BGN tofu chunks ranged from 39.15 (vinegar-tofu chunks) to 46.49 (0.6% GDL + 0.5 GA + 0.5 SA). The lightness of BGN tofu chunks made with vinegar was significantly ($p < 0.05$) lower compared to that of tofu chunks made with 0.6% GDL with and without gum Arabic and sodium alginate. Bambara groundnut tofu chunks prepared with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate were lighter in colour (L* = 46.49) compared to the tofu chunks prepared with 0.6% GDL (L* = 41.61) as observed in Figure 4.4. The addition of vinegar led to darker coloured tofu chunks (L* = 39.15). This suggested that the inclusion of gum Arabic and sodium alginate increased the lightness of the tofu, while other vinegar decreased it.

Table 4.6 Effect of gum Arabic and sodium alginate on Bambara groundnut tofu chunks[1]

	Colour parameter		
Tofu chunks	**Lightness (L*)**	**Redness/Greenness (a*)**	**Yellowness/Blueness (b*)**
0.6% GDL	41.61 ± 0.34^{a}	6.39 ± 0.41^{a}	16.25 ± 0.79^{a}
0.6% GDL + 0.5 GA + 0.5 SA	46.49 ± 0.23^{b}	8.43 ± 0.48^{ab}	17.03 ± 1.70^{a}
4% Vinegar + 0.5 GA + 0.5 SA	39.15 ± 0.53^{c}	8.83 ± 1.93^{b}	16.66 ± 1.02^{a}

[1]Values are mean ± standard deviation of triplicate analysis. Means within a column followed by different superscripts are significantly ($p \leq 0.05$) different. [2]GA: Gum Arabic; SA: Sodium alginate; GDL: Gluconolactone.

There was no significant difference in yellowness among all BGN tofu chunks. Yellowness ranged from 16.25 (GDL-tofu chunks) to 17.03 (0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate tofu chunks). Furthermore, tofu chunks made with 0.6% GDL combined with gum Arabic and sodium alginate appeared more yellow (Figure 4.4).The redness/greenness of BGN tofu chunks ranged from 6.39 (0.6% GDL-tofu chunks) to 8.83 (vinegar tofu-chunks).

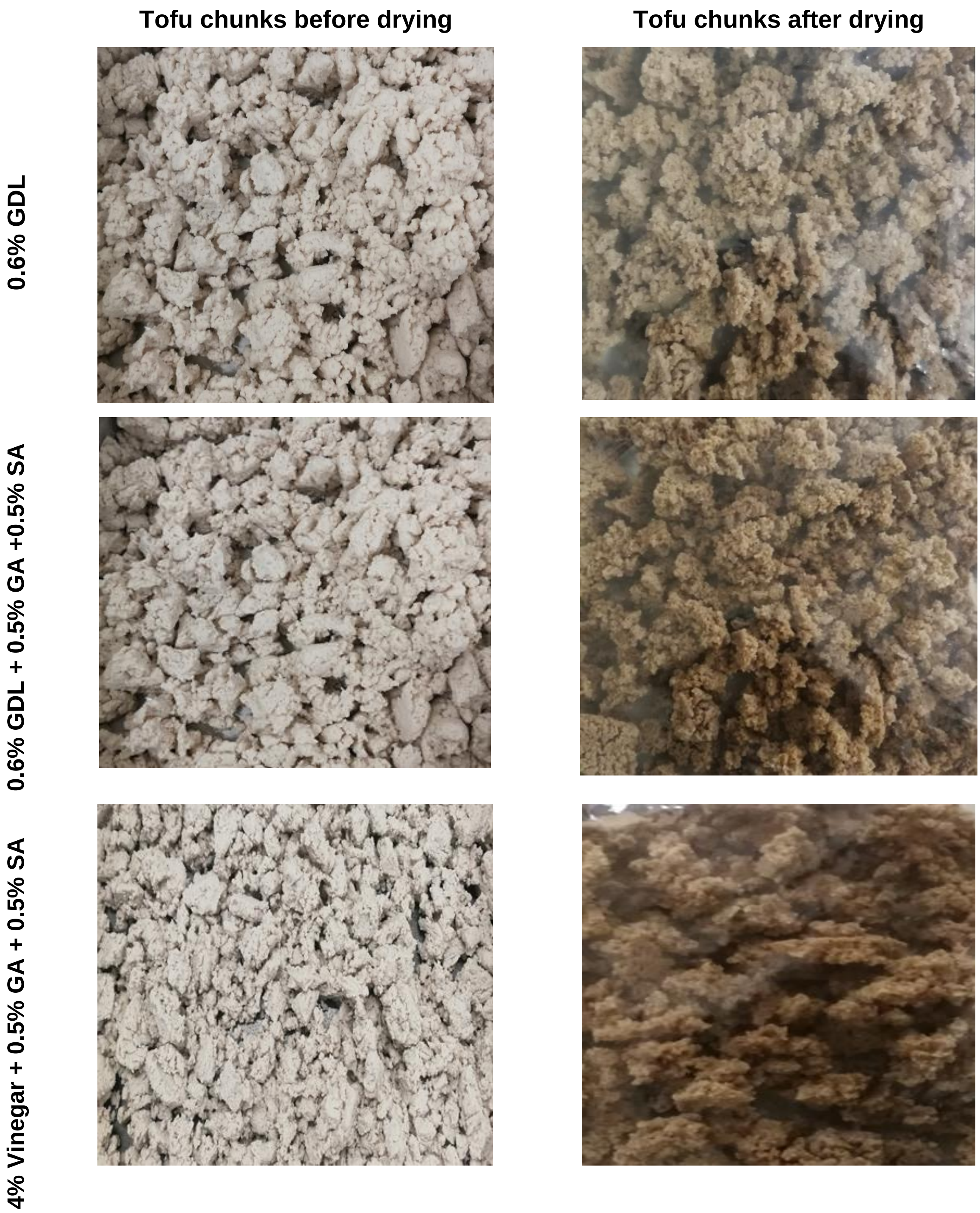


Figure 4.4: Bambara groundnut tofu chunks before and after drying.

The tofu chunks made with vinegar were more red compared to those made with 0.6% GDL + 0.5 gum Arabic + 0.5% sodium alginate, although the differences were not significant. These chromaticity changes can be attributed to Maillard reaction compounds, which result from the reduction of sugar and amino compounds in BGN or soybeans when heated. According to Baik

& Mittal (2003), heating tofu triggers the Maillard reaction, destroying active food enzymes and causing browning.

The findings of this study indicate that gum Arabic and sodium alginate have a greater effect on colour than the coagulants, as evidenced by a brighter appearance of BGN tofu chunks. The results of this study do not agree with those of Baik & Mittal (2003), who reported that coagulants had a greater influence on colour than hydrocolloids. Only gum Arabic and sodium alginate had a significant role in lightening the colour of tofu chunks. As such, the observed colour differences may be due to the Maillard reaction and not necessarily the choice of coagulant.

Wet tofu chunks were initially white to light grey. However, after the drying process, the chunks turned brown. The colour change can be attributed to various chemical reactions and Maillard browning that occur when proteins and sugars are exposed to heat. The Maillard reaction starts when reducing sugar react with an amino acid, forming glycosylamine. The glycosylamine then undergoes rearrangement to produce intermediate compounds (Schiff bases) (Xiang *et al*., 2021). Moreover, these compounds further react to form a range of complex molecules such as melanoids which contribute to colour and flavour of cooked food (Xiang *et al*., 2021). Tofu chunks made with vinegar + 0.5% gum Arabic + 0.5% sodium alginate were browner compared to those made with 0.6% GDL (Figure 4.4).

The presence of hydrocolloids, such as gum Arabic and sodium alginate, can influence the dispersion and reflection of light within the tofu matrix, leading to a visible colour change. This could be due to the ability of these hydrocolloids to interact with the proteins in the tofu, affecting light absorption and reflection properties (Zhang *et al*., 2023a). Additionally, the specific chemical and structural properties of gum Arabic and sodium alginate, as well as their interaction with other components in the tofu, can impact the overall colour of the BGN tofu chunks. Therefore, the use of gum Arabic and sodium alginate in tofu preparation can result in perceptible changes in the colour of the final product. The choice of additives can also influence colour changes during cooking or drying processes. Research by Ishiwatari *et al.* (2013) and Liu *et al.* (2022) showed that overheating proteins alters their physical properties, making the structure tougher. This is consistent with the well-known denaturation of proteins at high temperatures, which can alter texture and structure, causing changes in texture and taste. Furthermore, the particle size of tofu chunks affects their colour (Kim *et al*., 2019). Smaller, finer particles are more evenly distributed throughout the tofu chunks matrix, causing less disruption to the overall structure and resulting in a lighter appearance. In contrast, larger, coarser particles create more heterogeneity in the tofu, leading to darker spots or areas and reducing overall lightness. Therefore, using smaller, finer particles in tofu production helps maintain a lighter colour and appearance compared to larger, coarser particles, which tend to darken the tofu colour (Kim *et al*., 2019).

4.3.8 Sensory acceptability of Bambara groundnut tofu chunks

Table 4.7 shows the mean scores and standard deviations for various sensory attributes, including appearance, aroma, colour, taste, texture, and overall acceptability for tofu chunks prepared with 0.6% GDL, 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate and vinegar + 0.5% gum Arabic + 0.5% sodium alginate. There was a significant ($p < 0.05$) difference for appearance, colour, taste, texture, and overall acceptability of all tofu chunks. The appearance scores of tofu chunks ranged from 3.43 to 4.26 for tofu chunks made with vinegar + 0.5% gum Arabic + 0.5% sodium alginate and 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate, respectively. It was deduced that tofu chunks made with GDL were visually more appealing to the evaluators than those prepared with vinegar.

Despite variations in other attributes, the aroma remained consistent across the different samples. This due to the coagulants used which are highly acidic, and have a strong flavour. Therefore, rinsing curds before the pressing stage may reduce the acidic taste and flavour on the chunks.

The overall acceptability of BGN tofu chunks ranged from 3.55 (vinegar-tofu chunks) to 3.92 (0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate), indicating a moderately liked status. The addition of gum Arabic and sodium alginate was well-accepted by the panelists but did not significantly improve overall acceptability of the tofu chunks. The tofu chunks made with vinegar + 0.5% GA + 0.5% SA, received the lowest overall acceptability score (3.55).

The tofu chunks made with 0.6% GDL + 0.5% GA + 0.5% SA received the highest sensory rating for appearance, aroma, colour, taste, texture, and overall acceptability. This finding is consistent with Yasin *et al*. (2019), who reported a positive correlation between texture, taste, and overall acceptability of tofu. The sensory evaluation results highlight differences in attributes such as appearance, taste, and overall acceptability, which are linked to the choice of coagulants and hydrocolloids used in the tofu-making process. The BGN tofu chunks samples served to the panel are presented in Figure 4.

Table 4.7 Sensory qualities of Bambara groundnut tofu chunks as affected by coagulant, gum Arabic and sodium alginate[1]

Tofu chunks	Appearance	Aroma	Colour	Taste	Texture	Overall acceptability
0.6% GDL	3.83 ± 0.88[a]	3.94 ± 0.96[a]	3.75 ± 0.84[a]	3.56 ± 1.16[a]	2.96 ± 1.12[a]	3.58 ± 1.18[a]
0.6% GDL + 0.5% GA + 0.5% SA	4.26 ± 0.68[b]	4.09 ± 0.96[a]	4.21 ± 0.89[b]	3.89 ± 1.07[a]	3.34 ± 1.18[a]	3.92 ± 0.96[a]
4% Vinegar + 0.5% GA + 0.5% SA	3.43 ± 1.20[c]	3.98 ± 0.97[a]	3.57 ± 1.17[a]	3.58 ± 1.20[a]	3.28 ± 1.29[a]	3.55 ± 1.23[a]

[1]Values are mean ± standard deviation of triplicate analysis. Means within a column followed by different superscripts are significantly ($p \leq 0.05$) different. [2]GA: 0.5% Gum Arabic; SA: 0.5% Sodium alginate. [3]Sensory evaluation rating: Dislike very much (1); Dislike moderately; (2), Neither Like or Dislike (3); Like moderately (4); Like very much (5).

a

Figure 4.5: Sensory evaluation serving of Bambara groundnut tofu chunks prepared prepared with 0.6% GDL, 0.6% GDL + 0.5% GA + 0.5% SA and 4% vinegar + 0.5% GA + 0.5% SA.

4.4 Conclusions

The study highlighted the promising attributes of BGN tofu chunks, including their high protein content, improved amino acid composition, water absorption capacity, textural profile, bulk density, microstructure, colour and sensory properties when prepared with gum Arabic and sodium alginate. A mince-meat-like product with relatively high protein, and a well-balanced amino acid profile was successfully produced from BGN tofu. The tofu chunk made with 0.6% GDL had the highest moisture content (0.33%), while the formulation prepared with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate had the lowest moisture content (0.25%). The protein content was highest in the tofu chunks prepared with 4% vinegar + 0.5% GA + 0.5% SA (53.09%), indicating that this formulation may be particularly beneficial for protein enrichment. Tofu chunks offer a cost-effective source of nutrients to combat protein malnutrition and provide essential amino acids, aligning with the recommended levels set by the FAO and WHO, thus indicating their nutritional adequacy. The amino acid profile of BGN tofu chunks is notably abundant in essential amino acids, thus making them a promising dietary addition to improve overall protein intake and nutritional balance. Bambara groundnut tofu chunks exhibited maximum water absorption of 1.21% after soaking for 50 min, with increased soaking time enhancing water absorption. This increased absorption aids in speeding up chemical reactions during cooking and softening the texture of the tofu chunks. The compression parameters of BGN tofu chunks remained consistent across different coagulants and additives, suggesting that these factors did not significantly affect the tofu chunks' compression behaviour. Tofu chunks made with GDL were less dense thus suggesting less storage space and fast cooking. Tofu chunks coagulated with 0.6% GDL had a uniform, smooth surface with small holes, making them optimal for maximum compression compared to chunks prepared with vinegar. Additionally, the microstructure of chunks with GDL appeared more continuous and fine, and had a lower water absorption rate. Gum Arabic and sodium alginate significantly impacted the colour of tofu chunks, with these additives associated with a positive increase in all colour attributes compared to the coagulants alone. Sensory attributes, including appearance, taste, and overall acceptability, were influenced by the choice of coagulants and hydrocolloids utilised in the tofu-making process. Tofu chunks made with GDL + 0.5% gum Arabic + 0.5% sodium alginate exhibited higher hardness compared to vinegar + 0.5% gum Arabic + 0.5% sodium alginate, resulting in a chewy texture that provided a more preferred mouthfeel and an appealing flavor profile for the panelists. Bambara groundnut tofu chunks prepared with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate have good nutritional composition, desirable sensory and compression characteristics and this makes them a potential substitute for animal protein products.

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CHAPTER FIVE

GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 General Summary and Conclusions

This thesis reported the functionality of gum Arabic and sodium alginate on Bambara groundnut (BGN) (*Vigna subterranea* (L.) Verdc) tofu and tofu chunks. This study aimed to assess the effect of gum Arabic and sodium alginate on the textural, rheological and physicochemical properties of BGN tofu and its chunks. The objectives of the study were to optimise the concentration of gum Arabic and sodium alginate for optimal BGN tofu, establish its textural, rheological and physicochemical properties, characterise the functional properties and consumer acceptability of BGN tofu chunks. Tofu was produced from BGN milk extract.

The study was divided into two research chapters, (chapters three and four). The objective of chapter three was to establish the textural, physicochemical and rheological properties of BGN tofu as affected by coagulants (gluconolactone (GDL), vinegar and lemon juice) and hydrocolloids (gum Arabic and sodium alginate). A response surface I-optimal point exchange randomised design was used to determine the effect of gum Arabic (0.3, 0.4, 0.5%) and sodium alginate (0.3, 0.4, 0.5%) on the textural, physicochemical and rheological properties of BGN tofu. The textural properties (Instron), colour properties (HunterLab), expressible and entrapped water (centrifuge), proximates (AOAC), rheological properties (rheometer) and microstructure (scanning electron microscopy) were evaluated. These analyses confirmed that the optimal formulation for BGN tofu included gum Arabic and sodium alginate, each at 0.5%. This combination resulted in tofu with a firmer texture, a hardness of 9.11 N and a springiness of 11.53 mm. Tofu coagulated with GDL and vinegar were lighter in colour, as well as firmer and springier in texture, and had high protein content. These additives effectively reduced the amount of trapped water in the tofu, suggesting that the final product would possess desirable texture and extended shelf life. Tofu coagulated with GDL were more viscous, which enhanced its rheological properties. Vinegar-tofu exhibited a more uniform, smooth surface with fewer large pores, indicating a more compact and dense structure.

The objective of chapter four was to produce BGN tofu chunks and assess their functional and consumer acceptability through sensory evaluation, which included evaluation of appearance, colour, taste, aroma, texture, and overall acceptability. The proximate analysis (AOAC), amino acid profile (high-performance liquid chromatography), water absorption (centrifuge), textural properties (Instron), bulk density (measuring cylinder), microstructure (scanning electron microscopy) and colour properties (HunterLab) were studied. A mince-meat-like product with relatively high protein content and a well-balanced amino acid profile was successfully produced from BGN tofu. Vinegar-tofu chunks prepared with gum Arabic and sodium alginate had the highest protein content (53.09%). Among the essential amino acids, histidine had the highest concentration (2.59 g/100 g), while lysine had the lowest (3.29 g/100 g) in 0.6% GDL and vinegar tofu chunks. Bambara groundnut chunks demonstrated a greater absorption capacity of 1.21% after soaking in water for 50 min, suggesting a shorter cooking time. Although the compression parameters of BGN tofu chunks did not differ significantly from one another, 0.6% GDL tofu chunks had the highest (80.97 Kgf) compression force. Tofu chunks were denser than ground chunks, with vinegar-tofu chunks showing the highest density (0.73 g/mL).

The microstructure of tofu chunks coagulated with 0.6% GDL was uniform, featuring a smooth surface with small holes, making them optimal for maximum compression compared to those prepared with other coagulants. The absence of large holes in the microstructure images suggested a more compact structure of chunks. Tofu chunks made with 0.6% GDL and 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate were lighter than those coagulated with vinegar. The addition of gum Arabic and sodium alginate increased the lightness of the BGN tofu chunks, while the addition of vinegar, along with hydrocolloids, decreased lightness. Tofu chunks made with 0.6% GDL + 0.5% gum Arabic + 0.5% sodium alginate received the highest sensory scores compared to those coagulated with vinegar and 0.6% GDL alone. This suggested that the concentration of vinegar may need to be adjusted.

The following conclusions can be drawn from this study:

1. The optimal BGN tofu was produced using a combination of 0.5% gum Arabic and 0.5% sodium alginate.
2. Gum Arabic and sodium alginate can be successfully used to modify textural, physicochemical and rheological properties of BGN tofu.
3. The use of GDL as a coagulant and the addition of hydrocolloids like gum Arabic and sodium alginate contributed to superior textural, physicochemical and rheological properties of BGN tofu.
4. BGN tofu chunks with desirable functional and sensory qualities were produced from the optimal BGN tofu.
5. BGN tofu and its chunks are a cost-effective, high-protein, and nutritious option for consumers seeking plant-based protein sources.

5.2 Future Studies and Recommendations

Further studies could look into adjusting the concentration of vinegar used as a coagulant and ensure that the resulting curds are rinsed prior to pressing to achieve better sensory scores in the final BGN tofu chunks. Additionally, research is needed to explore the application of BGN tofu in various food systems such as plant-based burgers, meat substitutes, soups, and salads. Investigating how BGN tofu performs in these different food systems can help assess its versatility and potential benefits. Furthermore, examining the combined effect of vinegar and GDL on the textural and physicochemical properties of BGN tofu will provide valuable insights into optimising its quality.

ADDENDUMS

ADDENDUM A

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

Cape Peninsula University of Technology

Statement of Permission

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

Reference no.	212280341/04/2020
Surname & name	Chipeta, L.M.
Student Number	212280341
Degree	Master of Food Science and Technology
Title	Functionality of gum Arabic and sodium alginate on selected properties of Bambara groundnut tofu and its chunks.
Supervisor(s)	PROF VICTORIA ADAORA JIDEANI & DR YVONNE MAPHOSA
FRC Signature	
Date	2020 April 27

ADDENDUM B

Ethics approval letter

Cape Peninsula
University of Technology

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

Ethics Approval Letter **Reference no:** 212280341/04/2020

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

On 27 April 2020, the Faculty Research Ethics Committee of the Faculty of Applied Sciences granted ethics approval to Chipeta, L.M. for research activities related to a project to be undertaken for a degree (Master of Food Science and Technology) at the Cape Peninsula University of Technology.

Title of project:	Functionality of gum Arabic and sodium alginate on selected properties of Bambara groundnut tofu and its chunks.

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

1. Human subjects are included in the proposed study.
2. This permission is granted for the duration of the study.
3. Research activities are restricted to those detailed in the research proposal.
4. The research team must comply with conditions outlined in AppSci/ASFREC/2015/1.1 v1, CODE OF ETHICS, ETHICAL VALUES AND GUIDELINES FOR RESEARCHERS.

	27/04/2020 **Date**

ADDENDUM C

Book of abstracts – Research outputs presented at national Conferences.

2018 Postgraduate Conference

ECONOMIC GROWTH AND INTERNATIONAL COMPETITIVENESS (2)

Londiwe Chipeta 212280341

ORAL PRESENTATION

EFFECT OF PROCESSING PARAMETERS ON THE TEXTURE AND YIELD OF BAMBARA GROUNDNUT TOFU

The objective of this study was to investigate the coagulant type and coagulant concentration with a view to producing Bambara groundnut (BGN) tofu with greater yield and improved texture. Tofu was prepared using the vinegar and lime (lemon juice) as protein coagulant and Bambara groundnut flour (BGNF) as the source of protein. The prepared tofu was investigated for texture profile, colour, perce microstructure. The yield of tofu was expressed as grams of tofu per litre of BGN milk. Furthermore, the results were statistically analysed through the descriptive tests. Tofu produced from vinegar and salt with 4% concentration had a greater yield (34%) as compared to tofu made with lime and salt (32%). BGN tofu differed significantly ($p \leq 0.05$) for colour and textural characteristics. The colour characteristics ranged from 1.71 ± 0.40 to 1.08 ± 0.04 for redness (a^*), 7.15 ± 1.52 to 0.46 ± 0.06 for yellowness (b^*), 1.21 ± 0.05 to 73.52 ± 0.14 for lightness (L^*). The addition of salt increased the hardness of tofu made with lime from 10.21 ± 2.65 to 10.41 ± 2.09. There was no significant difference in the structural and network formation of protein on BGN tofu made with vinegar and lime. BGN tofu had an average of 11.93% protein and 6.33% fibre content.