EVALUATION OF PHYSICAL TRAITS AND CHEMICAL COMPONENTS ASSOCIATED WITH HARD-TO-COOK PHENOMENON IN BAMBARA GROUNDNUT (*Vigna subterranea* (L.) Verdc.)

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BY

Tan Xin Lin

School of Biosciences

Faculty of Science and Engineering

University of Nottingham Malaysia

ABSTRACT

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is a potential crop for future sustainable agri-food systems. However, utilisation of the crop is constrained by several factors, one of which is the hard-to-cook (HTC) phenomenon. The HTC seeds do not soften sufficiently after prolonged cooking time, thus demanding more energy for preparation. This, in turn, reduces the economic value and consumers acceptance of the pulse. The objective of this project was to explore the HTC traits in Bambara groundnut through the evaluation of physicochemical, microstructural, and technological properties to provide a basis to improve the processing efficiency and utilisation of the nutrient-dense pulse.

A screening study was first conducted to assess the variability and relationship between physical, microstructural, hydration and cooking characteristics among 12 Bambara groundnut genotypes. The physical traits of the seeds, which were characterised in terms of geometric, gravimetric, and seed coat properties, varied among genotypes. Thick seed coat (95.29-133.19 μm) and palisade layer (70.62-103.03 μm), compact cotyledon cells, narrow hilar groove, small tracheid bar, and occluded micropyle were among the factors contributing to the poor hydration behaviour of Bambara groundnut. During the soaking process, the seed coat, which exhibited moisture-dependent permeability, was the primary barrier to initial water uptake among the dry seeds. A sigmoidal model was applied to describe the hydration kinetics of the seeds. Three hydration parameters were subsequently estimated: (1) equilibrium moisture content (94.5-135.2 %), (2) hydration rate (0.095-0.272 h^{-1}), and (3) time to achieve half saturation (9.6-24.8 h). The cooking times (CTs) also showed genotypic variation, ranging from 70-208 mins and 38-120 mins for partially and fully hydrated seeds, respectively. The CT of fully hydrated seeds was not correlated ($p>0.05$) with any of the physical, microstructural and hydration kinetics parameters. It

was, however, significantly ($p<0.05$) correlated with leaching losses during soaking, supporting the cell membrane deterioration hypothesis.

The objective of the second stage of this study was to examine the differences in the characteristics of starch, protein, and cell wall materials between easy-to-cook (ETC) and hard-to-cook (HTC) genotypes. Two ETC genotypes (C_KARO and R_SONG; CT: 38-43 min) and two HTC genotypes (B_IPBB and N_ANAM; CT: 80-120 min) were selected for this study. Genotypic differences in cooking time could not be attributed to protein content and solubility. Additionally, the results of Fourier transform infrared spectroscopy indicated that there was no association between ease of cooking and molecular order of starch, secondary structure of protein, and molecular structure of cell wall materials. However, using a differential scanning calorimetry, a greater ($p<0.05$) thermal stability was observed among the HTC genotypes, as reflected by a higher thermal transition temperature and enthalpy of change. The HTC genotypes also exhibited a higher content of chelator-soluble pectin ($p<0.05$) and a lower content of water-soluble pectin ($p<0.01$) compared to the ETC genotypes, indicating the role of pectin solubility in strengthening intercellular adhesion and delayed cell separation during cooking.

In the final study, the response surface methodology was applied to identify the optimal soaking solution for Bambara groundnut genotype C_NAV4 to maximise the hydration capacity and cookability of seeds while minimising colour changes of the cooked seeds. Consequently, a soaking solution containing 0.25% $NaHCO_3$ + 0.14% Na_2CO_3 was selected. A comparative study was then conducted to assess the impact of salt solution on the hydration and cooking behaviour of the seed. The most notable effects arising from the presence of alkaline salt in the soaking solution were: (1) an improved hydration behaviour during soaking, as evidenced by a shorter lag time (τ = 8.1 h) and a faster hydration rate ($k = 0.211\ h^{-1}$) than that of distilled water-soaked seeds (τ = 10.9 h and $k = 0.181\ h^{-1}$, respectively), (2) a greater leaching loss throughout soaking and cooking processes, (3) a significant ($p<0.05$) decrease in the level of chelator-soluble pectin, and

(4) a 2.5-fold increase in the rate constant of cooking. These results indicate that the salt solution was effective in improving the hydration rate and shortening the cooking time of Bambara groundnut. The microstructural changes at various stages of cooking provided evidence for an association between cotyledon cell separation and texture softening of seeds.

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LIST OF ABBREVIATIONS

AIR	Alcohol insoluble residue
ANFs	Anti-nutritional factors
ANOVA	Analysis of variance
BGPI	Bambara groundnut protein isolate
CCRD	Central composite rotatable design
CDTA	Cyclohexane-trans-1,2-diamine tetra-acetic acid
CSP	Chelator-soluble pectin
CT	Cooking time
DoM	Degree of methyl-esterification
DSC	Differential scanning calorimetry
DW	Distilled water
dwb	Dry weight basis
EC	Electrical conductivity
FTIR	Fourier transform infrared spectroscopy
GalA	Galacturonic acid
HC	Hydration capacity
HS	Hard shell
HTC	Hard-to-cook
IVPD	In vitro protein digestibility
PME	Pectin methylesterase
RSM	Response surface methodology
SDG	Sustainable development goal
SEM	Scanning electron microscopy
SL	Solid loss
SS	Salt solution
WSP	Water-soluble pectin

LIST OF SYMBOLS

a*	CIELAB coordinate a*
b*	CIELAB coordinate b*
C*	Chroma
C(t)	Percentage of cooked seeds at time t
D_g	Geometric mean diameter
E	Mean relative deviation
h°	Hue angle
k	Rate of rehydration
k_c	Cooking kinetic constant
L	Length
L*	CIELAB coordinate L*
M_{eq}	Equilibrium moisture content
M(t)	Moisture content at time t
R^2	Coefficient of determination
SA_H	Hilum surface area
SA_S	Seed surface area
SSA_H	Specific hilum surface area
T	Thickness
T_c	Conclusion temperature of thermal transition
T_g	Glass transition temperature
T_o	Onset temperature of thermal transition
T_p	Peak temperature of thermal transition
V_C	Calculated seed volume
W	Width
ΔE*	Colour change/ colour difference
ΔH	Enthalpy change during thermal transition
τ	Time required to achieve half saturation during soaking
τ_c	Time needed to cook 50% of seeds

LIST OF EQUATIONS

Chapter 1: Background information

1.1 Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a legume indigenous to Africa and is cultivated across the semi-arid sub-Saharan region at the subsistence level (Azam-Ali *et al.*, 2001; Hillocks, Bennett and Mponda, 2012). The crop is resilient to climatic stresses and can yield on poor, marginal soils (Vurayai, Emongor and Moseki, 2011; Musa *et al.*, 2016). Its adaptability across different regions in Africa consisting of different soil types, temperature, photoperiods, pests, and diseases makes Bambara groundnut an important crop that can help to improve food security in sub-Saharan Africa. Besides, its nitrogen-fixing ability allows Bambara groundnut to be incorporated into intercropping and crop rotational systems to improve soil fertility (Alhassan and Egbe, 2013).

In addition to its beneficial agronomic traits, studies revealed superior nutritional profile for Bambara groundnut (Halimi *et al.*, 2019; Hussin *et al.*, 2020), which has earned its reputation of being a "complete food". Not only does the legume contain a well-balanced macronutrient composition (64% carbohydrate, 25% protein and up to 9% lipid; Halimi *et al.*, 2019), it is a rich source of micronutrients, such as magnesium, iron, zinc, and potassium. The crop is an important source of plant-based protein in areas where animal protein is scarce and is prized for its taste. However, the crop remains a subsistence crop, grown primarily by women farmers (Hillocks, Bennett and Mponda, 2012). The reasons for this are numerous but relate to difficulties with utilisation, and value chain constraints.

Hard-to-cook (HTC) phenomenon is one of the major hurdles to the utilisation, not only of Bambara groundnut, but of all legumes (Shehata, 1992). The HTC seeds require extensive cooking time and thus higher energy and water consumption to achieve palatability. This

becomes a major problem especially for areas where firewood is used for fuel or where water is scarce. The sensory and nutritional quality of seeds are also negatively affected as a result of seed hardening (Onayemi, Osibogun and Obembe, 1986; Tuan and Phillips, 1992). Hence, HTC not only undermines the economic potential of legumes, but can be deleterious to the food and nutritional security especially among the vulnerable populations in developing countries.

Both HTC and hard shell (HS) are textural defects associated with prolonged cooking of most pulses. Hard shell refers to the physical state of the seed, in which the impermeable seed coat is unable to imbibe water during soaking, thus preventing seed softening during cooking (Liu and Bourne, 1995). On the other hand, HTC seed cotyledon resists softening during cooking even after sufficient water imbibition. While the cookability could be a hereditary trait, factors such as growing environment and storage conditions could also induce HS and HTC (Jackson and Varriano-Martson, 1981; Wang *et al.*, 2017). In regard to storage condition, prolonged storage under high temperature and low humidity condition leads to HS, whereas HTC occurs under elevated temperature (>25^{o}C) and humidity (>65%) conditions (Liu and Bourne, 1995), that is, the ambient storage condition in the tropics.

1.2 Hypotheses for seed hardening mechanism

A number of mechanisms have been postulated to explain the causes of HTC phenomenon:

Pectin-cation-phytate model

Pectin, a polysaccharide composed mainly of D-galacturonic acids joined by α-1,4-glycosidic linkages, plays a major role in the composition of plant cell walls. It is also present between the cell walls in the middle lamella, where it acts as intercellular cement (Liu and Bourne, 1995). Some of the carboxyl groups (-COOH) in pectin are esterified with methanol into methyl ester form (-$COOCH_3$). Low-methoxyl pectin has a lower content (<50%) of esterified carboxyl groups and higher proportions of free carboxyl group, and vice versa for high-methoxyl

pectin (Chigwedere *et al.*, 2019). Since the free carboxyl groups can crosslink with divalent cations, the degree of methyl-esterification of pectin can influence the affinity of pectin for these ions.

Phytic acid is an antinutrient often found in the cotyledon of cereal and legumes, where it serves as a phosphorus reserve (Rousseau *et al.*, 2020). At physiological pH, the highly charged phosphate groups have a high tendency to chelate to mineral cations, especially Ca^{2+} and Mg^{2+}, and form stable, indigestible complexes (Mohan, Tresina and Daffodil, 2015).

The pectin-cation-phytate model is the most widely accepted postulated mechanism for the development of HTC (Reyes-Moreno, Paredes-López and Gonzalez, 1993; Gwala *et al.*, 2019): At cellular pH, phytate (the anion form of phytic acid) chelates to Ca and Mg cations to form phytate-cation complexes called phytin. During storage, enzyme phytase hydrolyses phytin into inorganic phosphate, which has a lower chelation potential, and liberates the divalent cations. Meanwhile, the enzyme pectin methylesterase removes the methyl esters of pectin and yields free carboxyl groups ($-COO^-$), which favour binding to divalent cations. Subsequently, the free divalent cations released from phytin migrate to the middle lamella and cross-link with the free carboxyl groups of the de-esterified pectin. The formation of insoluble Mg- or Ca- pectate complexes strengthen cell wall structure and cell-cell adhesion. This is evidenced by restricted cell separation and texture softening during cooking (Yi *et al.*, 2016; Chigwedere *et al.*, 2019).

This model has been confirmed by many researchers, who observed a reduction in water-soluble pectin and an increase in insoluble pectin in legume seeds over prolonged storage under adverse conditions (del Valle *et al.*, 1992; Shiga, Lajolo and Filisetti, 2004; Njoroge *et al.*, 2016). Additionally, studies found a negative relationship between phytate content and the cooking time of seeds (Martín-Cabrejas *et al.*, 1997; Galiotou-Panayotou, Kyriakidis and Margaris, 2008; Wainaina *et al.*, 2022). Being a powerful chelator to divalent cations, high phytate content

therefore prevents the formation of insoluble pectate. There are, however, discrepancies to this theory. Some studies reported lack of correlation between the phytate content and cooking time (Deshpande and Cheryan, 1986b; Alves *et al.*, 2021), and between the degree of pectin methylation and seed cookability (Liu, Phillips and Hung, 1992).

Cell lignification

Lignin is one of the constituents of cell walls and middle lamella that provides strength and rigidity to the cell. It is formed by oxidation and polymerisation of polyphenolic compounds. Protein degradation may occur during adverse storage, as evidenced by the accumulation of small polypeptides and the lower proportion of high molecular-weight protein in storage-induced HTC seeds (Hohlberg and Stanley, 1987). Protein degradation produces free aromatic amino acids. The presence of the lignin substrates, i.e. the free phenolic compounds and aromatic amino acids, promotes lignin synthesis, thereby strengthening middle lamella. Although lignin build-up has been observed in HTC seeds using Transmission Electron Microscopy (Hincks and Stanley, 1987), this hypothesis remains contentious as studies on the effect of storage on lignin and polyphenol contents showed inconsistent results (Mafuleka *et al.*, 1993; Martín-Cabrejas *et al.*, 1997; Nasar-Abbas *et al.*, 2008).

Phenolic compounds

Besides lignin, other phenolic compounds have been suggested as possible contributor to HTC development (Srisuma *et al.*, 1989). Studies suggested the involvement of phenolic compounds (tannins and hydroxycinnamic acid) in binding to pectin to form insoluble pectate, thereby reinforcing cell adhesion (Garcia *et al.*, 1998; Mubaiwa *et al.*, 2019). Stanley (1992) hypothesised that tannin migration from seed coat to cotyledon during storage may lead to formation of stable complexes with proteins, thereby contributing to HTC. Martín-Cabrejas *et al.* (1997) demonstrated a reduction in total polyphenols and non-tannin polyphenols in HTC bean,

and attributed the observation to the polymerisation of phenolic compounds into insoluble, high molecular weight products.

Cell membrane damage

According to this hypothesis, cell membrane breaks down under high temperature and humidity storage as a result of lipid degradation (Shehata, 1992). This is accompanied by denaturation of proteins (transport proteins and receptors) and destruction of protein channels that regulate the movement of cations, thus leading to increased membrane permeability and decreased cell turgor pressure (Liu and Bourne, 1995). Loss of membrane integrity in aged seeds is evidenced by increased solute leakage during soaking (Jones and Boulter, 1983; Wacu *et al.*, 2015; Buzera, Kinyanjui and Ishara, 2018).

Proteins and starch

In the presence of water, thermal treatment of seeds leads to starch swelling and gelatinisation, and protein denaturation. Studies reported lower degrees of protein denaturation and starch gelatinisation in cooked HTC seeds (Hincks, Mccannel and Stanley, 1987; Garcia-Vela and Stanley, 1989a; Garcia and Lajolo, 1994). The development of HTC also influenced the enthalpies and temperatures of starch gelatinisation and protein denaturation (Garcia-Vela and Stanley, 1989a; Garcia and Lajolo, 1994). These observations led to the postulation that prolonged storage under adverse conditions reduces the hydration capacity of HTC seeds, consequently leading to restricted starch gelatinisation and protein denaturation during cooking, thus rendering the seeds hard to cook.

Another hypothesis was proposed by Liu *et al*. (1992b), who reported lower water solubility and thermal stability of protein in HTC seeds. The authors attributed the observation to protein insolubilisation during storage due to lowered cellular pH in aged seeds. This causes the soluble proteins to coagulate more readily and denature at a temperature lower than that of starch

gelatinisation. Consequently, the coagulated proteins act as a physical barrier to water absorption and starch swelling, thereby impeding starch gelatinisation.

Multiple mechanism

This hypothesis proposes that during prolonged storage, biochemical reactions such as phytate and pectin hydrolysis, lignin synthesis, protein degradation and insolubilisation, and lipid peroxidation may occur concurrently or sequentially, causing changes in the microstructure, physical characteristics and chemical composition of the seed (Hincks and Stanley, 1986; Liu and Bourne, 1995). The synergism of these reactions may amplify their effects, eventually leading to HTC phenomenon.

1.3 Practical approaches to address HTC

Since it is clear that storage conditions contribute significantly to the phenomenon, the most obvious solution is to improve storage conditions of the seeds, such as by reducing storage temperature or by using appropriate packaging (Aguilera and Rivera, 1990). However, this may require energy and incur extra cost, which may be infeasible for resource-poor areas, particularly in those developing countries located in the tropics. Breeding cultivars that are less prone to HTC development is yet another preventive measure against the HTC defect, however it takes a long time to assess and understand the interactions between cultivar and the growing conditions (location, soil and climate) before the desirable traits can be selected (Stanley *et al.*, 1990).

In addition to the preventive strategies, appropriate food processing methods could be applied to encourage utilisation of hardened seeds. Soaking hard seeds in salt solution has been shown to be a low-cost approach to reduce cooking time (de León, Elías and Bressani, 1992; Ávila *et al.*, 2015; Mubaiwa *et al.*, 2019). Sodium chloride, sodium bicarbonate, and alkaline rock salt are among the commonly used salts. Salts are believed to reduce cooking time through several actions: increase pectin solubilisation by displacing divalent cations bound to pectin;

increase protein solubility by altering pH of soaking medium; and enhance water uptake and thermal penetration by improving bean porosity (Shehata, 1992). These actions are influenced by the type, concentration and monovalent-to-divalent ratio of salt (de León, Elías and Bressani, 1992; Ávila *et al.*, 2015).

1.4 Rationale and objectives

The recent events of global climate change, geopolitical tensions, and pandemic outbreaks underscore the urgent need for a concerted effort to ensure the sustainability of food systems. Diversification of food supplies through increased utilisation of minor crops, such as Bambara groundnut, is one approach to achieve this goal. However, despite its potential to contribute to regional and global food and nutritional security, the uptake of Bambara groundnut is challenged by several factors, one of which is its HTC characteristics. Therefore, enhanced understanding on this phenomenon could enable the development of potential solutions, which in turn may contribute to increased utilisation of the crop.

Numerous efforts have been made to address the HTC phenomenon in commonly consumed legume seeds, resulting in publications that have successfully pinpointed possible factors associated with the HTC phenomenon (summarised in Section 1.2). Although these works were performed mostly on the genus *Phaseolus*, useful information can be drawn from these findings and be translated into guideline for application on Bambara groundnut.

This project seeks to:

i. Assess the variability and correlation of the physical, rehydration and cooking characteristics across different Bambara groundnut genotypes. HTC seeds are undesirable as the preparation process becomes inconvenient and increases energy consumptions. Thus, the cookability of the seed is an important trait for breeders. Understanding the

inherent variability and the relationships between these parameters that may be useful in predicting the seed cookability for future breeding programme.

ii. Determine the contents and characteristics of certain chemical composition (pectin, protein, and starch) of Bambara groundnut and associate the compositional differences with respect to the ease of cooking. These chemical components are among the compositional factors that have been proposed to be associated with HTC (Section 1.2). Therefore, additional information may enhance understanding on their roles in influencing seed cookability.

iii. Evaluate the effect of salt solutions on the cookability of Bambara groundnut in order to improve the processing quality and utilisation of Bambara groundnut. The application of salt solution during soaking or cooking of legume seeds has been found to increase water uptake rate and the tenderness of cooked seeds (Section 1.3). Such improvements in technological properties of Bambara groundnut, which are desirable for consumers and processors, may therefore promote the utilisation of the crop.

The schematic illustration of the objectives and the workflow addressing each objective are summarised in Figure 1.

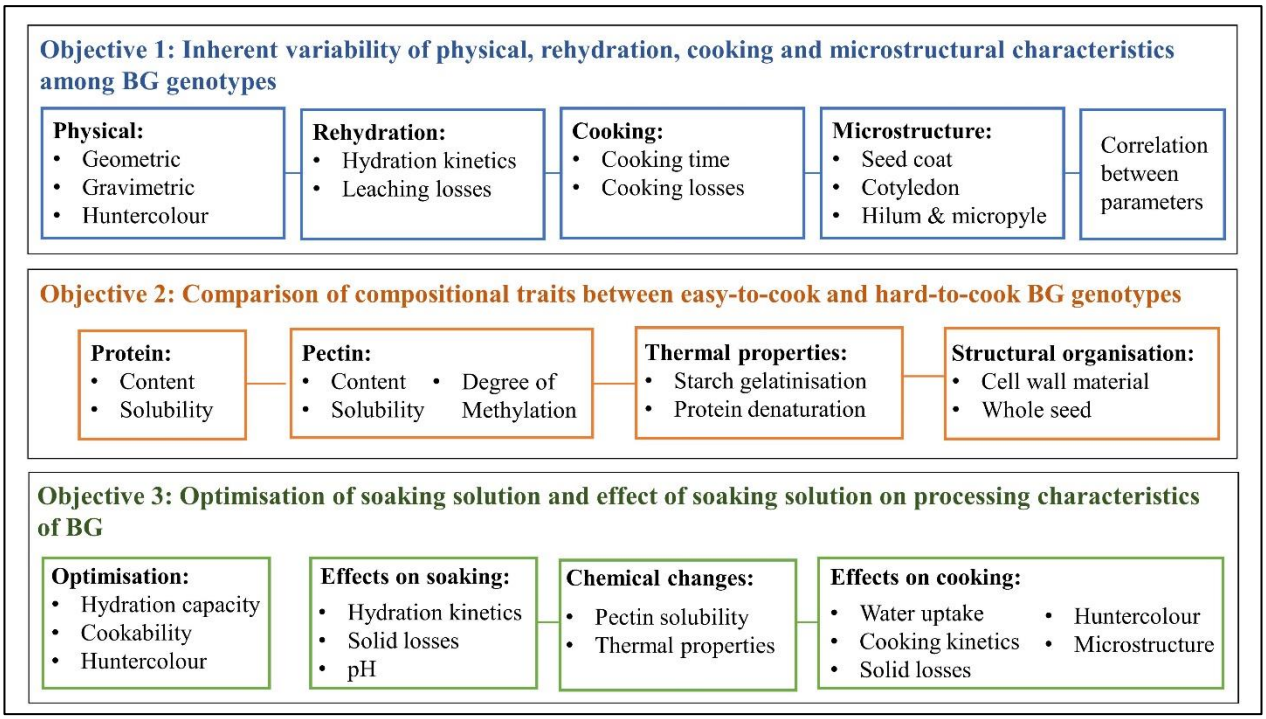

Figure 1 An overview of the thesis chapters of this project.

This thesis is comprised of seven chapters. Chapter 1 outlines the background information of the HTC phenomenon. Chapter 2 is a literature review on the Bambara groundnut, with a focus on its potential contribution to the future sustainable food systems (published as "Bambara groundnut: an underutilised leguminous crop for global food security and nutrition" (Tan *et al.*, 2020)). Chapter 3 describes the general materials and methods to conduct the experiments. The three objectives stated in Figure 1 are addressed in Chapters 4, 5, and 6, respectively. The final chapter discusses the general conclusions, limitations of the current study and recommendations for future work.

Chapter 2: Literature review – Bambara groundnut: an underutilised leguminous crop for global food security and nutrition

Published review article (Tan XL, Azam-Ali S, Goh EV, Mustafa M, Chai HH, Ho WK, Mayes S, Mabhaudhi T, Azam-Ali S and Massawe F (2020) Bambara Groundnut: An Underutilized Leguminous Crop for Global Food Security and Nutrition. Front. Nutr. 7:601496. Doi: 10.3389/fnut.2020.601496)

2.1 Introduction

Eliminating hunger requires an adequate intake of energy and nutrients. Providing a healthy diet requires a food-based approach to improving diet and nourishing individuals. Despite the rich agrobiodiversity on Earth, humanity has evolved to rely on a few crops for nourishment. The last few decades have seen a global increase in the supply of dietary energy, through increased yield and production worldwide (FAO, 2019). However, this does not translate to the nutritional quality of the food we consume, nor does it ensure availability, accessibility and affordability of food to vulnerable populations. The recent decades have seen an increase in prevalence of hunger, childhood overweight and adult obesity (FAO *et al.*, 2020). Should we continue with our current production and consumption patterns, we are unlikely to achieve the UN Sustainable Development Goal (SDG) of Zero Hunger by 2030 (FAO *et al.*, 2020). Factors such as population growth, urbanisation and changes in dietary pattern towards resource-intensive foods are driving the demand for increased food production (FAO, 2018). Pest and disease outbreaks, resource depletion, regional conflicts and climate change are set to further undermine the capacity of the food system and exacerbate the situation (FAO, 2018; FAO *et al.*, 2020). To meet the SDG of zero hunger by 2030 and to end malnutrition in all its forms, the target is to increase the availability and accessibility to nutrients, not just calories. Adoption of a diversified healthy diet, with emphasis on affordable nutrient-rich plant-based foods such as fruits, vegetables, whole grains and legumes can contribute to sustainable food and nutrition security (EAT-Lancet Commission, 2019; FAO *et al.*, 2020) and to the achievement of SDG2.

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a legume indigenous to Africa and is cultivated across the semi-arid sub-Saharan Africa region (Hillocks, Bennett and Mponda, 2012). It is a hardy crop and has been recognised as an important nutritious food source when food is scarce (Mbosso *et al.*, 2020). This could be attributed to its climate-smart features, including its ability to fix nitrogen and to grow under adverse environmental conditions such as poor soils and drought (Mayes *et al.*, 2019; Paliwal *et al.*, 2020). This nutrient-dense legume is sometimes termed a "complete food" due to its balanced macronutrient composition. Bambara groundnut contains approximately 64.4% carbohydrate, 23.6% protein, 6.5% fat, and 5.5% fibre and is rich in minerals (Halimi *et al.*, 2019). It is relatively underutilised compared to major cash crops and has often been associated with small-scale, subsistence farming, with women being the major producers and processors (Mubaiwa *et al*., 2018; Mbosso *et al.*, 2020). The utilisation constraints of Bambara groundnut include the knowledge gap in improved seed system, agronomic practices, processing and utilisation. Genetics, agronomy and nutritional aspects of Bambara groundnut and its food uses have been recently reviewed by other authors (Oyeyinka and Oyeyinka, 2018; Halimi *et al.*, 2019; Mayes *et al.*, 2019; Nwadi, Uchegbu and Oyeyinka, 2020). This paper gives an overview of the value chain and discusses the potential role of Bambara groundnut in closing the gaps in the food system to ensure sustainability of food and nutritional security.

2.2 Closing the food supply gap through improved production of Bambara groundnut

Bambara groundnut is thought to have its centre of origin somewhere between West and Central Africa (Temegne *et al.*, 2018). It is grown widely in sub-Saharan Africa and is also present at low levels in Thailand, Malaysia and Indonesia (Mayes *et al.*, 2019). Higher preference for Bambara groundnut has been observed in dry regions prone to drought (Mubaiwa *et al.*, 2018). This is possibly linked to its ability to produce reasonable yields under such conditions,

hence acting as a safety net for farmers. Bambara groundnut production in Africa is reported to be approximately 0.3 million tonnes annually with an average of 0.85 t ha^{-1}, although the yield potential is reported to be over 3 t ha^{-1} (Hillocks, Bennett and Mponda, 2012; Nedumaran *et al.*, 2015). Nigeria is regarded as the largest producer of Bambara groundnut with a mean production of 0.1 million tonnes, followed by Burkina Faso 44,712 tonnes, and Niger 30,000 tonnes (Hillocks, Bennett and Mponda, 2012).

2.2.1 Genetic diversity and implications – traditional landraces vs modern varieties

Most germplasm planted by famers is in the form of landraces with high genetic variability. This is reflected by the wide variations in morphological (Ntundu *et al.*, 2006) and nutritional (Halimi *et al.*, 2019) traits across Bambara groundnut landraces. Genetic variability can act as a form of insurance for farmers as some members of the landrace population can provide local adaptation, stress tolerance and yield stability (Dwivedi *et al.*, 2016), thus giving farmers a higher chance of obtaining some form of seed yield in times of drought or other stresses.

High genetic variability observed in landraces also lends itself to high potential for crop improvement in Bambara groundnut. Most of the currently grown improved varieties of Bambara groundnut are generally landraces selected for improved yield, seed and flour quality, and drought tolerance (Karikari, 2000). Bambara groundnut is usually sown as a minor crop, intercropped with other staples, by small holder African farmers for household consumption (Hillocks, Bennett and Mponda, 2012). For this reason, coupled with low current market demand for the crop, yield stability is seen to be a more important aspect for landrace improvement than grain yield in order to ensure food security. Besides, the genetic diversity preserved in the gene pool allows some of these accessions to be developed into high protein and high oil cultivars (Halimi *et al.*, 2019), suggesting its potential in contributing to nutritional security in the region. Breeding improvement efforts of the promising landraces could consequently lead to improved

profitability of the crop as well as adoption of the crop in diverse Bambara groundnut growing regions.

2.2.2 Productivity traits and agroecological adaptation

Variation in Bambara groundnut productivity has been attributed to agro-ecological factors, such as climate (Berchie *et al.*, 2016), soil fertility (Tyoakoso, Toungos and Babayola, 2019), water availability (Chai, Massawe and Mayes, 2016) and daylength (Kendabie *et al.*, 2020). Nonetheless, it has been shown to exhibit adaptability across different regions under diverse growing conditions. For instance, the crop exhibits tolerance to soil acidity and low soil fertility (Uguru and Ezeh, 1997), as well as adaptability to the tropical degraded acidic soils (Musa *et al.*, 2016). Despite being classified as a facultative short-day crop for pod set (Kendabie *et al.*, 2020), many landraces have adapted to regions with a broad range of daylengths. Physiological experiments have also revealed good recovery qualities when the crop is subjected to water stress (Vurayai, Emongor and Moseki, 2011). Its yield is reported to be well above those of chickpea and similar to groundnut cultivars under comparable drought stress conditions (Collino *et al.*, 2000; Mwale, Azam-Ali and Massawe, 2007). This indicates that selection for drought tolerance is key considering that the crop is generally cultivated in arid to semi-arid regions of sub-Saharan Africa. Limited studies indicated that, although field drought conditions reduce the seed yield in Bambara groundnut, there is no effect on the nutritional quality of the seed (Brough and Azam-Ali, 1992). This trend has been observed in limited landraces and also in common bean (*Phaseolus vulgaris* L.) (Smith *et al.*, 2019), but further studies on Bambara groundnut would be required to confirm this hypothesis.

Some areas where Bambara groundnut is grown have poor soils that are lacking in nitrogen. Most farmers in those regions do not apply synthetic fertilisers to their crops because the costs are often prohibitive (Nweke and Emeh, 2013). Bambara groundnut, as other nodulating legumes,

can fix atmospheric nitrogen to replenish soil nitrogen, hence making it a potential companion crop for intercropping and rotational systems. It is often intercropped with cereals and root crops that can provide a significant amount of the calorie intake (Ncube *et al.*, 2007). Its incorporation into crop rotation cycles can help to maintain soil fertility and break the cycles of pests and diseases, which is advantageous to resource poor farmers who might generally be unable to afford fertilisers and pesticides (Alhassan and Egbe, 2013). Incorporation of Bambara groundnut in intercropping system with maize has been shown to increase the productivity of maize (Egbe, Alhassan and Ijoyah, 2013), indicating its potential contribution towards agrobiodiversity and subsequently food security. Varying rates and amount of nitrogen fixation have been observed for different Bambara groundnut accessions (Musa *et al.*, 2016), and the enhancement of symbiotic nitrogen fixation was indicated to potentially increase its yield (Dakora and Muofhe, 1997). The variability of nitrogen fixing capacity among Bambara groundnut landraces offers room for cultivar improvement and a positive correlation with yield would be an ideal scenario for the breeder.

2.3 Closing the nutrient gap through enhanced utilisation of Bambara groundnut

2.3.1 Bambara groundnut as a *"complete food"*

There is a growing trend towards increased consumption of plant-based diets, resulting in a need for more plant-based protein foods. Bambara groundnut is the obvious crop to consider. It serves as an important source of essential nutrients in areas where animal protein is scarce (Boye, Zare and Pletch, 2010). The nutritional composition of Bambara groundnut has earned it the reputation of being a complete food, and this will be explored further in subsequent sections.

2.3.1.1 Balanced macronutrient composition

Carbohydrates

Carbohydrates are the most abundant macro-nutrient in Bambara groundnut, accounting for up to 64.4% of the total dry weight of the seed (Halimi *et al.*, 2019). The majority of the carbohydrate fraction are complex oligosaccharides and polysaccharides, of which starch accounts for up to 49.5% of the total carbohydrates. The reported starch content of Bambara groundnut seeds varies considerably (22% to 49.5% of dry seed weight), depending on genetic and environmental factors, stage of maturation and method of analysis (Oyeyinka and Oyeyinka, 2018). Amylose represents 19.6-35.1% of the total starch content, while the rest of the constituents consist primarily of amylopectin and a small quantity (1-2%) of protein, lipid and ash (Oyeyinka and Oyeyinka, 2018). Raw Bambara groundnut has a higher proportion of slowly digestible starch (SDS) and resistant starch (RS) than rapidly digestible starch (RDS) (Oyeyinka, Singh and Amonsou, 2017), implicating poor digestibility. Nonetheless, cooking can substantially increase the RDS fraction (Oyeyinka, Pillay and Siwela, 2019), thereby improving digestibility and carbohydrate availability.

Protein

The protein content of Bambara groundnut ranges from 9.6-40% (Nwadi, Uchegbu and Oyeyinka, 2020), with an average value of 23.6% (Halimi *et al.*, 2019). This variation is also attributed to differences in genetic background, growing conditions, and analytical techniques used for estimation (e.g. nitrogen conversion factor) (Boye, Zare and Pletch, 2010; Mubaiwa *et al*., 2018). Storage proteins are the predominant protein fractions in Bambara groundnut, of which vicilin (7S) is reported to be the major constituent, followed by legumin (11S) (Adebowale, Schwarzenbolz and Henle, 2011).

High protein content is a desirable trait in foods, but the importance of protein quality, which is determined by both amino acid composition and protein digestibility, should not be overlooked. Variability in amino acid profile between cultivars of Bambara groundnut is evident.

In general, most studies report glutamic acid to be the most abundant amino acid in Bambara groundnut, suggesting its potential to be isolated for use as a flavouring agent. Out of the essential amino acids, leucine and lysine are present at a higher concentration while methionine is the lowest (Yao *et al.*, 2015; Adebiyi, Njobeh and Kayitesi, 2019; Hussin *et al.*, 2020). Phenylalanine, valine, histidine and isoleucine were also reported to be present in high concentrations, while tryptophan has been found to be the limiting amino acid (Yao *et al.*, 2015; Oyeyinka, Pillay and Siwela, 2019). Its lysine-rich, methionine-poor composition makes Bambara groundnut a good complementary protein source to cereals, which are often deficient in lysine but rich in methionine (Boye, Zare and Pletch, 2010). The *in vitro* protein digestibility (IVPD) of raw and cooked Bambara groundnut varies between 70-81% and 82-87.5%, respectively (Mazahib *et al.*, 2013; Oyeyinka, Pillay and Siwela, 2019). The increase of IVPD after cooking is attributed to the destruction of heat labile anti-nutritional factors (ANFs) and fragmentation of native proteins into smaller polypeptides, subsequently improving enzyme accessibility and protein bioavailability.

Lipids

There is considerable variation (1.4% and 9.7%) in the reported values of lipid content in Bambara groundnut (Adebowale, Schwarzenbolz and Henle, 2011; Yao *et al.*, 2015). The majority of fatty acids in Bambara groundnut are unsaturated, predominated by oleic and linoleic acids (omega-6) (Yao *et al.*, 2015; Adeleke *et al.*, 2017). Palmitic acid is the third most abundant fatty acid, and linolenic acid (omega-3) is present at a low concentration. While having 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 uses should be taken into consideration when selecting the desirable trait of lipid composition.

2.3.1.2 Rich in essential micronutrients

Minerals

The most abundant minerals in Bambara groundnut are potassium, magnesium, phosphorus, zinc and iron (Oyeyinka, Pillay and Siwela, 2019; Hussin *et al.*, 2020; Qaku, Adetunji and Dlamini, 2020). Halimi *et al.* (2019) reported that the levels of these minerals were higher than those found in commonly consumed legumes such as chickpea and mung bean, but they vary by cultivar and growing conditions. The presence of ANFs in the seeds can adversely affect the bioavailability of the minerals. Gwala *et al.* (2020) reported that the concentration and bio-accessibility of calcium, magnesium, iron and zinc in Bambara groundnut seeds were influenced by factors such as storage period, processing method, location of mineral in the seeds (testa or cotyledons), and the degree and strength of mineral chelation. Despite being a relatively good source of these minerals, it is unlikely that the dietary needs of individuals can be met through consumption of Bambara groundnut alone.

Phytochemicals

Bambara groundnut seeds contain phytochemicals such as flavonoids and tannins. These compounds are usually found in the seed coats and are more abundant in seeds with dark or red coloured seed coats. A positive correlation between darkness of seed coat and total phenolic compounds has been established (Tsamo, Ndibewu and Dakora, 2018). Mubaiwa *et al.* (2019) reported an abundance of the flavonoids epicatechin and catechin in raw and cooked red seed, respectively. Catechin and epicatechin can polymerise to form proanthocyanidins, also known as condensed tannins, which have been associated with nutraceutical properties, such as antioxidant, cardioprotective, antitumour, and neuroprotective properties (Rauf *et al.*, 2019). Antioxidant properties have been reported in brown and red Bambara groundnut seeds, levels of which were comparable to commonly consumed legumes, but inferior to the powerful antioxidant ascorbic

acid (Nyau *et al.*, 2015, 2017). Despite the positive health outcomes associated with consumption of phytochemical compounds, their antinutritional implications should not be overlooked.

2.3.1.3 Other important functional properties

Dietary fibre

Bambara groundnut contains appreciable level of dietary fibre in the form of resistant starch (RS) and non-starch polysaccharides. The concentration and composition of dietary fibre are influenced by maturity stage and processing methods (Yao *et al.*, 2015). Total dietary fibre content of Bambara groundnut ranges from 1.4% to 10.3%, of which insoluble fibre represents a higher fraction than soluble fibre (Halimi *et al.*, 2019). The relatively high proportions of SDS, RS and dietary fibre in Bambara groundnut reduces the rate of digestion and lowers the postprandial glycaemic response, rendering Bambara groundnut a low glycaemic index (GI) food (Oyeyinka, Singh and Amonsou, 2017). From one point of view, it is advantageous to encourage the consumption of low GI foods as these confer numerous health benefits e.g., lowering postprandial blood glucose and insulin levels, regulating appetite, and reducing the risks of obesity and other non-communicable diseases. Conversely, the increased consumption of flatus-causing non-starch polysaccharides has been associated with irritable bowel (Mohan, Tresina and Daffodil, 2015). More importantly, from a nutritional security point of view, non-digestible dietary fibres (e.g. lignin) can bind to minerals and form physical barrier during digestion and absorption, thus reducing the bioavailability of essential minerals (Rousseau *et al.*, 2020).

2.3.2 Processing of Bambara Groundnut to increase nutritive value and utilisation

2.3.2.1 Antinutritional factors (ANFs)

In common with other legumes, several ANFs have been identified in Bambara groundnut. Their presence can negatively affect the digestion and bioavailability of essential

nutrients. The commonly reported ANFs in Bambara groundnut include condensed tannins, phytic acid, and trypsin inhibitor. Condensed tannins are mainly located in the testa and are more abundant in the darker coloured seeds (Nti, 2009). Despite having an antioxidant capacity, these polyphenolic compounds can form indigestible complexes with dietary minerals, starch and proteins, thereby reducing their bioavailability (Mohan, Tresina and Daffodil, 2015; Unigwe *et al.*, 2018). Binding with proteins can inhibit the activity of digestive enzymes. Tannin compounds can also impart bitterness and astringency to the food (Rauf *et al.*, 2019), thereby affecting palatability. Phytic acid is more abundant in the seed cotyledon, where it serves as a phosphorus reserve for the plant (Rousseau *et al.*, 2020). At physiological pH, the highly charged phosphate groups have a high tendency to chelate to mineral cations and form stable, indigestible complexes (Duodu and Apea-Bah, 2017). Phytic acid can also crosslink with dietary proteins, starch and digestive enzymes, thus impairing the bioavailability of nutrients (Mohan, Tresina and Daffodil, 2015; Rousseau *et al.*, 2020). However, it is worth noting that phytic acid has been reported to exhibit antioxidant and anticancer properties, suggesting its potential health promoting properties (Mohan, Tresina and Daffodil, 2015). The major enzyme inhibitor reported for Bambara groundnut is trypsin inhibitor (Duodu and Apea-Bah, 2017). Inhibition of protease can negatively affect protein digestion and subsequently impede its absorption. Furthermore, low trypsin level can result in increased pancreatic secretory activity, thereby causing pancreatic hypertrophy (Adeleke *et al.*, 2017). The reported levels of ANFs among different Bambara groundnut cultivars vary widely (condensed tannins 0.0011-18.61 mg/g; phytic acid 1.10-15.11 mg/g; trypsin inhibitor 0.06-73.40 TI mg g^{-1}). These differences are attributed to genetic and environmental factors, as well as extraction and analytical methods (Duodu and Apea-Bah, 2017; Unigwe *et al.*, 2018).

Some forms of dietary fibres are also considered to have antinutritional properties. Pectins can bind to metal cations such as calcium, zinc and iron, which, not only reduces mineral

bioavailability, but affects the cookability of the legume (Rousseau *et al.*, 2020). Raffinose and stachyose, the flatus-causing alpha-oligosaccharides, are also present in Bambara groundnut (Adeleke *et al.*, 2017; Duodu and Apea-Bah, 2017). Other ANFs such as oxalate, hydrogen cyanide and saponins have also been detected in Bambara groundnut (Ndidi *et al.*, 2014; Adeleke *et al.*, 2017; Tsamo, Ndibewu and Dakora, 2018).

Certain food processing methods are effective at lowering the ANFs and this will be discussed in the following section. It is possible that the inherent levels of ANFs present in raw beans could be reduced by plant breeding (Bouis and Welch, 2010), which would be advantageous for improved utilisation of the legumes and in their contribution to enhanced nutritional security. However, gains made in improving the nutritional value through reduction of the anti-nutritional compounds, may be lost through increased susceptibility to pests and diseases during production and subsequent storage of the seeds. This is because these components are plant secondary metabolites that provide some resistance to stress, pests, and pathogens, therefore, reducing the levels may result in a compromised defence system (Bouis and Welch, 2010).

2.3.2.2 Traditional processing methods

If not eaten fresh, Bambara groundnut is dried post-harvest for long term storage. Drying is an effective food preservation technique to prolong the storage period and ensure food availability during food shortages (FAO, 1997). Prior to consumption, the dry seeds are either rehydrated by soaking in water or milled in the dry form into flour. Most of the pre-treatments, or processing have an impact on the nutritional, sensory and functional properties of the seeds. Traditional processing of Bambara groundnut involves basic equipment and can be carried out at the household level. Some of these processes have the potential to be mechanised and industrialised to improve the cost effectiveness, process efficiency and product uniformity, while

creating employment opportunities and providing income for rural people (FAO, 1997). The following section describes some of the traditional, often essential, processing stages of Bambara groundnut, and the impact on nutritional, processing and eating quality.

Dehulling

The seed coat, or testa, is sometimes separated from the cotyledons before further processing. Since a high proportion of the antinutritional components are present in the testa, dehulling can improve the digestibility and nutritional value of the seeds, in particular through increased mineral and protein availability (Oyeyinka *et al.*, 2017; Adebiyi, Njobeh and Kayitesi, 2019). Removal of the testa also reduces the dietary fibre content (Gwala *et al.*, 2020; Rousseau *et al.*, 2020), which can have both negative and positive implications, depending upon the nutritional status of the consumer. With respect to sensory attributes, removal of the highly pigmented seed coats, which are rich in tannins and fibres, has been shown to improve the appearance, texture and taste of Bambara groundnut products (Nti, 2009). Other implications of dehulling include increased leaching of minerals during soaking and cooking, which negatively impacts on the nutritional quality, and shortened fermentation time which is advantageous from a utilisation point of view (Adebiyi, Njobeh and Kayitesi, 2019).

Milling

Dried Bambara groundnut can be ground into flour to improve its versatility (Nwadi, Uchegbu and Oyeyinka, 2020). However, at the small scale, milling is laborious and time consuming due to a phenomenon described as ‘hard-to-mill’ (Mubaiwa *et al*., 2018). The disruption of cell wall structure, through milling or other abrasive processing activity, can increase the availability and digestibility of nutrients, starch and protein in particular (Do and Singh, 2018). Increased interaction between starch, protein and cell wall materials also results in

structural and functionality changes (Enwere and Hung, 1996). From a negative point of view, milling could increase interactions between minerals and ANFs, thus reducing their bioavailability (Raes *et al.*, 2014). In terms of food security, the hard-to-mill attribute is advantageous in that it allows dried seeds to be stored for very long periods as they are impervious to water, and resistant to pest and insect attack. However, from the utilisation aspect, these inherent difficulties incur increased energy costs for processing and may deter potential end users from choosing Bambara groundnut as a raw material, despite its nutritional and agronomic advantages.

Soaking

After drying, Bambara groundnut seeds are typically rehydrated by soaking in water for 12-24 hours before cooking. Soaking also has positive and negative impacts on the nutritional value of the seeds, primarily through leaching of nutrients and ANFs into the soaking water (Mazahib *et al.*, 2013; Rousseau *et al.*, 2020). The rate and degree of leaching are influenced by the binding strength of the biomolecules to the intracellular matrix (Gwala *et al.*, 2020), which can be manipulated by the temperature and pH of the soaking liquid. Studies reported higher loss of trypsin inhibitor and tannins in Bambara groundnut during hot water soaking, but the reverse was observed for phytate and oxalate (Barimalaa and Anoghalu, 1997; Adegunwa *et al.*, 2014). Soaking also facilitates the subsequent processing of Bambara groundnut. Increasing soaking temperature (up to 60^{o}C) improved water absorption rate and dehulling efficiency, and reduced dehulling loss (Enwere and Hung, 1996). Regarding functional properties, pre-soaking Bambara seeds before milling results in flour with higher foam capacity and improved pasting properties (Adegunwa *et al.*, 2014; Mubaiwa *et al.*, 2018). From a food and nutritional security perspective, soaking is a low cost, low-energy processing stage that can significantly improve the utilisation of Bambara groundnut.

Germination/ Malting

The nutritional value of Bambara groundnut seeds can be manipulated by germination. The process of soaking followed by sprouting for up to 72 hours (James *et al.*, 2018) reduces the carbohydrate and lipid content of the sprouts (Lyimo, Berling and Sibuga, 2004), while at the same time enhancing the protein content, amino acid profile and IVPD (Adeleke *et al.*, 2017; Awobusuyi and Siwela, 2019). Reduction of ANFs, such as tannins, trypsin inhibitor, oxalate, oligosaccharides and saponin, is due to leaching during soaking (Lyimo, Berling and Sibuga, 2004; Adeleke *et al.*, 2017). Sprouting is also beneficial to the dehulling process (Barimalaa and Anoghalu, 1997) as the seed coat splits open during sprouting.

Fermentation

Fermentation is another traditional, low-technology processing option that can be used to enhance the nutritional value of Bambara groundnut. Typically, the process involves soaking of the whole seeds, followed by dehulling, cooking, and wrapping in banana leaves before fermenting for about four days (Adebiyi, Njobeh and Kayitesi, 2019). Starter cultures may sometimes be added (Qaku, Adetunji and Dlamini, 2020) to enhance the fermentation. The positive impacts of fermentation are a breakdown of the flatus-forming non-digestible oligosaccharides and polysaccharides into digestible simpler carbohydrates and a reduction in ANFs and phenolic content (Adebiyi, Njobeh and Kayitesi, 2019). Bambara groundnut milk, an aqueous extract of the seed, can be fermented to prepare yoghurt. The yoghurt produced is reported to have a higher protein content and digestibility and a lower phytate content compared to Bambara groundnut milk (Pahane *et al.*, 2017).

Boiling

Bambara seeds are often cooked in excessive water for variable periods of time until the desired texture is attained. In common with the other treatments, genetic variability, physicochemical properties, age of the seed and storage conditions can affect the time taken to reach the desired end point. In the presence of water, thermal treatment leads to starch swelling and gelatinisation, protein denaturation, solubilisation of water-soluble pectins, and eventually cell separation (Do and Singh, 2018). The effects of boiling on nutritional quality of Bambara groundnut vary with cultivar, pre-treatment applied, and the length of cooking time. However, most studies report that boiling has a positive impact on nutritional quality through the destruction of ANFs (Nti, 2009; Adegunwa *et al.*, 2014; Ndidi *et al.*, 2014) and improved *in vitro* digestibility of starch and protein (Mazahib *et al.*, 2013; Oyeyinka, Pillay and Siwela, 2019). It is likely that improved digestibility of protein is due to destruction of ANFs, which are more susceptible to wet-heat than dry-heat, thereby releasing protein bound to them; whereas improved starch digestibility is due to the disruption of starch granules, consequently increasing amylolytic attack and hydrolysis.

The various processing stages applied to Bambara groundnut, at small and larger scale, have positive and negative implications for its food and nutritional security. The presence of various ANFs and the indigestible nature of the raw seeds means that processing is an essential stage for all legumes. From a food and nutritional security point of view, it is important to select a combination of processes that enhance the digestibility of the macro-nutrients, while at the same time minimising losses of both macro-nutrients and minerals. When selecting or recommending particular processing methods, it is essential to consider the energy costs of each one, as these can be prohibitive to the utilisation of the crop by resource-poor end users.

2.3.2.3 Traditional food uses

Bambara groundnut is commonly consumed as snack food after roasting or boiling (Murevanhema and Jideani, 2013; Mubaiwa *et al.*, 2018). The seeds and the flour have also been used to produce a myriad of traditional foods in different parts of Africa (Figure 2) (Nti, 2009; Ndidi *et al.*, 2014; Mubaiwa *et al.*, 2018; Bultosa *et al.*, 2020; Mbosso *et al.*, 2020). During the preparation of local delicacies, Bambara groundnut is often paired with cereals such as maize and millet (Mbosso *et al.*, 2020), which is beneficial in improving protein quality. In Nigeria, the popular '*okpa*' (steamed Bambara groundnut pudding), which is made from Bambara groundnut flour and red palm oil, plays an important role in contributing to dietary protein and vitamin A intake among school children (Ayogu *et al.*, 2017).

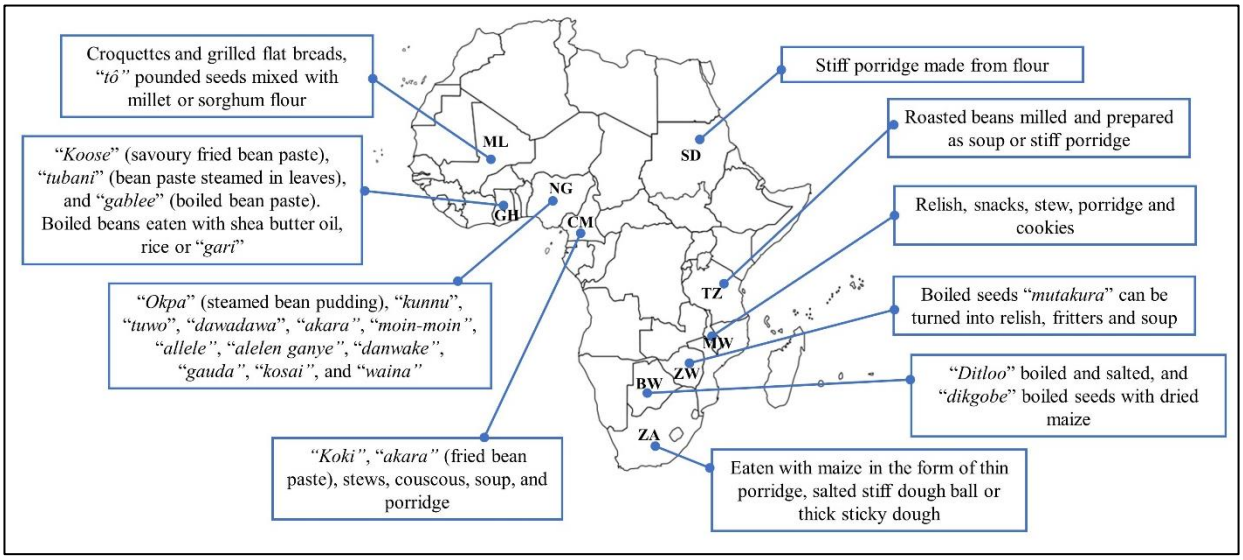

Figure 2 Traditional delicacies in different African countries (Mali (ML); Ghana (GH), Nigeria (NG); Cameroon (CM); South Africa (ZA); Botswana (BW); Zimbabwe (ZW); Malawi (MW); Tanzania (TN); and Sudan (SD)) prepared from Bambara groundnut.

2.3.3 Advanced processing technologies and potential food uses of Bambara groundnut

2.3.3.1 Advanced processing technologies

In addition to the traditional low-cost processing technologies, which are essentially used to make the seeds edible and fit for consumption, more advanced forms of processing can be employed to improve nutritional quality, modify the physicochemical characteristics, and expand the range of value-added products for increased utilisation. Irradiation, infrared heating, and autoclaving are among these more advanced techniques. Unlike traditional processing techniques, which can be employed at household level, these technologies require more sophisticated equipment and therefore are carried out on a larger scale.

Despite being a non-thermal processing method, gamma-irradiation causes starch degradation or pre-gelatinisation in Bambara groundnut, thus reducing the cooking time of the seeds (Falade and Adebiyi, 2015). Shorter cooking time could also be due to increased cell wall permeability to water and/or heat as a result of damaged microstructure (Celik *et al.*, 2004). Irradiation also affects protein structure and conformation, thus altering the functionality of the flour (Falade and Adebiyi, 2015).

Infrared heating (micronisation) can also result in reduction in cooking time of Bambara groundnut (Ogundele and Emmambux, 2018), which not only helps to save energy and water, but also has knock-on positive impacts on the retention of nutrients. This instant heating process, which causes starch gelatinisation and protein denaturation, could enhance the utilisation of Bambara groundnut seeds and flour for production of a diverse range of convenience products such as partially-cooked seeds or instant flour (Ogundele and Kayitesi, 2019).

The use of high-pressure heating, via autoclaving, is useful for improving the nutritional value of the cooked seeds. The application of heat under high pressure is effective at deactivating

ANFs, thus increasing the digestibility of proteins and starch (Adeleke *et al.*, 2017). The high temperature process can reduce the functionality of proteins, as measured by a reduction in foaming and emulsifying properties (Adegunwa *et al.*, 2014), which will limit the use of Bambara groundnut in food applications where emulsification and/or the ability to form a stable foam are essential attributes. From a nutritional security point of view however, any process that can improve the nutritional quality through improved digestibility and removal of ANFs, at the same time as reducing the cooking time and energy requirements, is a beneficial process.

2.3.3.2 Bambara groundnut in processed foods

Advanced processing technologies and enhanced insights into food science, coupled with increased consumer demand for convenience food have led to increased availability and accessibility to processed foods at lower prices (Sharif, Zahid and Shah, 2018). While processed foods offer numerous advantages to consumers such as convenience, diverse food choices and stability of food supply (Sharif, Zahid and Shah, 2018), the ultra-processed foods are often stripped of nutrients and are energy dense. Excessive consumption of empty calories can have negative impacts on health. To replace the micro-nutrients that are removed by processing, public health initiatives make the fortification of some food commodities with vitamins and minerals mandatory in most countries. Increasingly, alternative ingredients are being used to fortify staple foods, rather than relying on premix vitamins and minerals. This approach confers additional benefits to the consumer and producer – it offers a more holistic approach to food fortification or enrichment, utilising otherwise underutilised plant species, and diversifying the diet with more than just the specific mineral or vitamin. Bambara groundnut has the potential to serve as an ingredient for food fortification due to its affordability, versatility, nutritional quality and sensory acceptability.

Nwadi *et al*. (2020) recently reviewed studies on the application of Bambara groundnut flour in a number of products including snacks and pastries, breakfast cereal and pasta, traditional foods, composite flour, complementary food, and milk and yoghurt. To summarise, most studies reported that the protein content of the Bambara groundnut flour-incorporated products increased with the supplementation level, but sensory attributes were negatively affected. This paper discusses a few points that were not covered by Nwadi *et al*. (2020) review.

Staple foods: Breads and wheat noodles

Bread is one of the staple foods consumed on a global scale. It is commonly made from wheat flour, which is deficient in lysine. Complementing with legume flour or extracted legume protein can therefore improve its nutritional quality. However, consumers have come to expect leavened bread to have an airy texture, which is the result of gas being trapped inside a gluten network. Like other legumes, Bambara groundnut flour lacks gliadin and glutenin, which are crucial for the formation of gluten. Inclusion of Bambara groundnut flour into bread dough therefore has an adverse impact on the texture of the bread and this will limit its amount that can be used to substitute wheat flour. Reports on the use of Bambara groundnut flour in bread highlight impacts on protein weakening, increased dough development time, decreased dough consistency, stability and extensibility, water absorption, and loaf volume (Abdualrahman *et al.*, 2012; Erukainure *et al.*, 2016).These observations were attributed to an impaired development of the gluten network due to dilution of gluten. Nonetheless, the dough rheology and physical characteristics of Bambara groundnut flour-incorporated dough can be improved by addition of active surfactants such as pectin and emulsifiers (Ajibade and Ijabadeniyi, 2019), thereby improving the organoleptic properties of breads.

Wheat noodles are staple in many Asian countries and are gaining popularity in other parts of the world. Gluten is also important in the production of noodles, but not as critical as for

a leavened bread. Wheat-based noodles are increasingly being fortified with ingredients such as spinach, pumpkin, and sweet potato to improve the nutritional value. Bambara groundnut flour has been included in wheat-based noodles with varying degrees of success (Chude *et al.*, 2018; Hussin *et al.*, 2020).

In general, addition of Bambara groundnut flour into bread and noodles improved the protein quality, reduced ANFs and increased mineral contents (Abdualrahman *et al.*, 2012; Hussin *et al.*, 2020). The level of substitution of wheat flour could be the key to consumer acceptability. An appropriate substitution level can help to ensure improved nutrition without compromising consumer acceptability (Hussin *et al.*, 2020), thus making these products a promising food vehicle to combat nutrient deficiency. An alternative, more promising option for developing this area is to change consumer expectations, by marketing Bambara groundnut enriched loaf/ noodle as a novel and nutritious alternative product (Hussin *et al.*, 2020).

Snacks: Crackers/ Biscuits/ Extruded products

Popular snack foods, such as crackers, biscuits and extruded products, are typically made from wheat, rice or maize. They are potential foods for fortification programmes targeting school children and adult alike. Studies on the inclusion of Bambara groundnut flour in these products showed variable results in terms of their physical and functional properties (Chima and Fasuan, 2017; Ogunmuyiwa *et al.*, 2017; Yeboah-Awudzi *et al.*, 2018). These variations could be due to different formulations and production methods used, which might also explain the differences in nutritional and sensory qualities among these products. In order to maximise the potential of Bambara groundnut-enriched snacks in improving nutritional security of the population, local consumer preferences should be taken into consideration during product development.

Complementary/ Weaning foods

Traditional weaning foods in Africa are often prepared from low-cost but highly accessible ingredients such as cereals, roots, tubers and legumes (Samaila *et al.*, 2018; Makame *et al.*, 2019). They are often nutrient poor, characterised by high levels of starch, fibre and antinutrients, but inadequate levels of essential amino acids and micronutrients. Inappropriate processing methods also lead to poor texture and nutrient bioavailability (Makame *et al.*, 2019). Several studies have successfully developed complementary foods containing Bambara groundnut and reported enhanced nutritional quality: Bambara groundnut-enriched maize '*ogi*' showed increased protein, ash and fat contents and high consumer acceptability (Afolabi *et al.*, 2018); banana and fermented Bambara groundnut flour mix at 60:40 ratio showed comparable nutritional quality to commercial infant formula (Ijarotimi, 2008); and maize-Bambara groundnut complementary food fortified with micronutrients showed acceptable micronutrient levels to meet infant daily requirement (Uvere, Onyekwere and Ngoddy, 2010). To conclude, its low-cost, nutrient-dense features allow Bambara groundnut to be a viable alternative ingredient in enriching infant food products.

Beverages: Milk and Fermented drink

Inaccessibility of dairy milk in some countries, specific health related dietary requirements and the trend towards plant-based diets are factors that are driving the surge in demand for vegetable milk (Falade *et al.*, 2015; Murevanhema and Jideani, 2015). Bambara groundnut has the potential for the production of vegetable milk and yogurt (reviewed by Murevanhema and Jideani (2013)). The development of shelf-stable spray dried milk powder with acceptable hydration properties has been reported (Hardy and Jideani, 2018). Several reports refer to the nutritional and/or sensory properties of Bambara groundnut milk and yogurt (Katungwe, Mwangwela and Geresomo, 2015; Murevanhema and Jideani, 2015; Pahane *et al.*,

2017). Overall, it can be concluded that despite its high nutrient content, there remains works to be done to optimise the sensory and physicochemical properties of Bambara groundnut milk to gain wider consumer acceptance.

Amahewu, or *mahewu*, is a popular fermented drink traditionally made by fermenting sorghum or maize flour. It is non-alcoholic and is sometimes used as a weaning food (Qaku, Adetunji and Dlamini, 2020). However, it is nutrient poor and is often characterised as lacking essential amino acids (Awobusuyi and Siwela, 2019). Substituting the conventional ingredients with Bambara groundnut reduced phytate content while improving its protein quality and sensory acceptability (Awobusuyi and Siwela, 2019; Qaku, Adetunji and Dlamini, 2020).

2.3.3.3 Bambara groundnut as functional ingredient

In addition to providing essential nutrients, both the starch and protein of Bambara groundnut have functional properties, which may find wide application for food and non-food uses. Understanding and improving the functional properties of Bambara groundnut starch and protein isolate may increase the potential application and end-use of the crop, which *may* translate into an increased demand for the crop and benefits to the producer. However, it is prudent to point out that the relationship between increased utilisation and producer benefits is far from simple – it is complex, fraught with issues of sovereignty and equity, and with no guarantee of improved livelihoods for the producer.

Native starch

Starch is one of the most widely used and adaptable polysaccharides. In addition to providing energy, within the food industry, starch is used variously as a thickener, gelling agent, stabiliser, humectant; and for non-food uses such as a replacement for polystyrene and plastic in disposable packaging material, plates and cutlery, to name but a few. To fully maximise the

potential application of Bambara groundnut starch, it is essential to understand the structure and physicochemical properties of the native starch.

The composition, physicochemical properties and modification of starch has been reviewed by Oyeyinka and Oyeyinka (2018). Bambara groundnut starch granule is characterised by spherical, polygonal, irregular or oval shaped granules with smooth surface and a diameter from 6 to 35 μm (Kaptso *et al.*, 2015; Oyeyinka and Oyeyinka, 2018). Both the major starch constituents, the amylose and amylopectin fractions, influence its physicochemical and functional properties, which in turn affect its application.

Bambara groundnut starch has poor swelling capability compared to conventional starches such as potato, corn and cereal starches (Oyeyinka and Oyeyinka, 2018). This could be attributed to its relatively high amylose content, which results in a more rigid granular structure and therefore restricted swelling. The swelling power of Bambara groundnut starch increases with temperature, peaking at 80-90ºC, and decreasing thereafter (Gulu, Jideani and Jacobs, 2019). This temperature range corresponds to its gelatinisation temperature range, beyond which the starch granules rupture and the contents leach out, leading to inhibition of water uptake and swelling ability (Hoover, 2001). The peak gelatinisation temperature and enthalpy of gelatinisation of Bambara groundnut starch are higher than most of those reported for cereal and tuber starches (Joye, 2018), indicating its thermal stability. Bambara groundnut starch also exhibit a relatively high pasting temperature that is comparable with other legumes (Hoover, 2001), while its pasting viscosities showed huge variations (Oyeyinka and Oyeyinka, 2018). This could be due to differences in cultivar, experimental condition, for instance starch concentration and purity, and the analytical equipment used, thus making it difficult to compare the results.

Its poor functionality e.g. low swelling capacity and poor pasting properties gives the native starch limited applications as a functional ingredient. Possible application may include products in which restricted swelling and high thermal stability are desirable.

Modified starch

Native Bambara groundnut starch can be modified to improve and diversify its behavioural characteristics. Modifications can be made physically or chemically. Physical modification, which is considered a safer modification approach, is associated with alteration of the starch granules by heat application (Joye, 2018). Heat treatment causes intragranular molecular reorganisation of starch, thus leading to variable effects such as increased gelatinisation and pasting temperatures, and reduced swelling power, solubility and pasting viscosity (Oyeyinka and Oyeyinka, 2018). On the other hand, chemical modifications alter the starch structure through introduction or formation of new functional groups (Joye, 2018), thereby affecting its physicochemical properties. Different modification methods (oxidation, acetylation and carboxymethylation) can have variable effects on the solubility, swelling capacity, pasting properties, and water and oil absorption capacities of Bambara groundnut starch (Adebowale, Afolabi and Lawal, 2002; Afolabi, 2012). Bambara groundnut starch can also be modified by forming complexes with other components, such as lipids and cyclodextrin. This leads to increased thermal stability and the resultant starch paste displays higher ability to withstand shear stress with a lower tendency to retrograde (Oyeyinka *et al.*, 2017; Gulu, Jideani and Jacobs, 2019).

These studies indicate that, with proper selection of modification techniques, Bambara groundnut starch has a great potential to be used as a functional ingredient in food applications such as improving viscosity, mouthfeel, adhesion, freeze-thaw stability. Reduced digestibility of lipid-modified starch (Oyeyinka *et al.*, 2017), which could be due to formation of type-4 resistant

starch (Fuentes-Zaragoza *et al.*, 2010), could provide important nutritional functionality for diabetic patients or in weight management program.

Bambara groundnut protein isolate (BGPI)

Bambara groundnut protein can be extracted and used as a functional ingredient in a number of foods. The reported protein content in BGPI ranged from 81.4% to 92.8% (Kudre, Benjakul and Kishimura, 2013; Kaptso *et al.*, 2015). Its solubility is pH dependent and has been shown to be higher than mung bean and black bean protein isolates (Kudre, Benjakul and Kishimura, 2013). Besides, BGPI displays high thermal stability, which is comparable with mung bean, black bean and soy protein isolates (Adebowale, Schwarzenbolz and Henle, 2011; Kudre, Benjakul and Kishimura, 2013). Studies reported variable results on the physicochemical properties (water and oil absorption capacities, gelation capacity, and foaming and emulsifying properties) of BGPI (Arise *et al.*, 2017; Adeleke, Adiamo and Fawale, 2018; Alabi *et al.*, 2020). The differences in functional properties of BGPI can be explained by several factors, including the amino acid composition (Kudre, Benjakul and Kishimura, 2013), extraction method (Adebowale, Schwarzenbolz and Henle, 2011), extraction condition (Mune Mune, Bouba and Minka, 2015), and drying condition (Mune Mune and Sogi, 2016).

Since the physicochemical properties of BGPI can be altered and improved by various methods, BGPI has the potential to be a useful functional ingredient, especially for those avoiding animal-based products. BGPI exhibits high trypsin inhibitor activity (Kudre, Benjakul and Kishimura, 2013), which is undesirable from a nutritional quality point of view, but can be exploited as a preservative for fish products e.g. surimi. It can act as protease inhibitor to lower proteolysis and delay texture softening of surimi when applied at an appropriate level (Oujifard *et al.*, 2012). The hydrolysates of BGPI have been shown to exhibit potent antioxidant activities, which may find application in food preservation or as a functional food (Arise *et al.*, 2016). The

bioactive peptides were also found to inhibit renin and angiotensin-converting enzyme, two components known to be associated with hypertension (Arise *et al.*, 2017).

2.3.4 Enhanced production and consumption of Bambara groundnut as food: challenges and recommendations

The utilisation of Bambara groundnut is challenged by several factors, as summarised in Figure 3. This section focuses on three of the constraints, namely the cookability, bottlenecks in the value chain and safety issues, and discusses the possible solutions to these challenges.

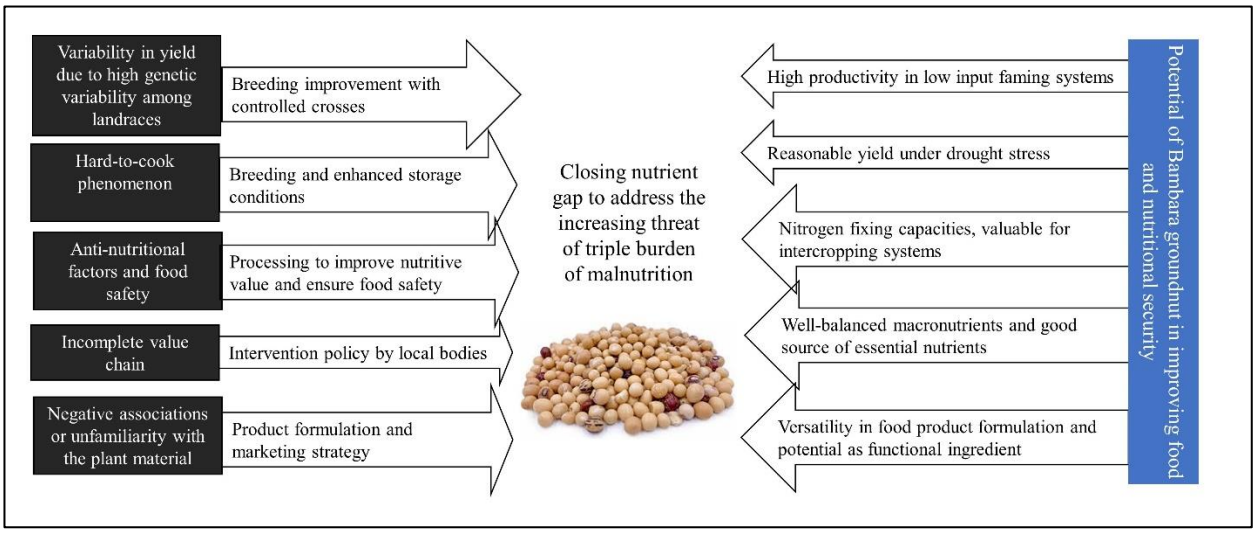

Figure 3 An overview of the challenges and opportunities of utilising Bambara groundnut in addressing food security and nutrition.

2.3.4.1 Hard-to-cook phenomenon

The hard-to-cook (HTC) trait in Bambara groundnut is one of the major hurdles to its utilisation (reviewed by Mubaiwa *et al.* (2017)). The HTC feature is associated with legume cotyledon resistance to softening during cooking, resulting in a prolonged cooking time to attain a desirable texture. Development of the phenomenon can be hereditary (Reyes-Moreno, Paredes-López and Gonzalez, 1993), and it can also be induced by extended storage under elevated temperature (>25°C) and humidity (>65%), that is, the ambient storage conditions in humid tropic

areas (Gwala *et al.*, 2019). The pectin-cation-phytate model is the most widely accepted postulated mechanism for the development of HTC (Reyes-Moreno, Paredes-López and Gonzalez, 1993; Liu and Bourne, 1995): Pectin is present in middle lamella where it acts as intercellular cement. Phytic acid, being a powerful chelator for cations, is usually bound to divalent cations especially Ca^{2+} and Mg^{2+}. During storage, phytase hydrolyses the phytate-cation complex, hence liberating the divalent cations; meanwhile, pectin methylesterase removes the methyl-esters of pectin, thus producing free carboxyl groups ($-COO^-$), which favour binding to the free divalent cations. The formation of insoluble Mg- or Ca- pectate complexes strengthens cell wall structure and cell-cell adhesion, thereby restricting cell separation and bean softening during cooking (Chigwedere *et al.*, 2019). Mubaiwa *et al.* (2019) also suggested the involvement of phenolic compounds (tannins and hydroxycinnamic acid) in binding to pectin to form insoluble pectate, thereby reinforcing cell adhesion. Another popular hypothesis is cell lignification, which postulates that protein degradation may occur during storage. The crosslinking between the freed aromatic amino acids and free phenolic compounds in the cell leads to lignin synthesis and causes cell wall toughening (Reyes-Moreno, Paredes-López and Gonzalez, 1993). Besides, other mechanisms, e.g. lipid peroxidation (Richardson and Stanley, 1991), protein solubilisation (Liu, McWatters and Phillips, 1992), cell membrane damage and ion redistribution (Jones and Boulter, 1983; Hincks, Mccannel and Stanley, 1987), might also occur during storage, soaking and cooking. The synergism of these reactions may amplify the effects, eventually leading to HTC phenomenon (Liu and Bourne, 1995).

HTC phenomenon is reported to have negative impacts on the nutritional quality of Bambara groundnut. Gwala *et al*. (2020) found that ageing decreased *in vitro* bio-accessibility of calcium and magnesium in Bambara groundnut seeds. Prolonged cooking of aged seeds also reduced the levels of minerals (Mg, Fe and Zn; Gwala *et al.*, 2020) and protein quality (Tuan and Phillips, 1992). The level of HTC and degree of cooking also influenced starch digestibility of

HTC Bambara groundnut (Gwala *et al.*, 2019). Additionally, hardening of beans not only reduces the eating quality, but also increases fuel and water consumption during cooking (Mubaiwa *et al.*, 2018). This becomes a major problem especially for areas where firewood is used for fuel or where water resources are limited. Consequently, the HTC phenomenon is not only inconvenient, it increases the cooking cost and also poses challenges for sustainability, which may limit its potential uptake on a larger scale (Gwala *et al.*, 2019).

Possible solutions to address the HTC defect include improving the storage conditions and breeding cultivars that are less prone to HTC (Reyes-Moreno, Paredes-López and Gonzalez, 1993). The former may require energy and incur additional costs, which may be infeasible for resource-poor areas, while the latter may take several generations of cross breeding. Simple low-cost processing techniques, such as soaking hard seeds in salt solution, has been demonstrated to reduce the cooking time (de León, Elías and Bressani, 1992; Ávila *et al.*, 2015; Mubaiwa *et al.*, 2019). Sodium chloride, sodium bicarbonate, and alkaline rock salt are among the commonly used salts. Salts are believed to reduce cooking time through several actions: increased pectin solubilisation by displacing divalent cations bound to pectin; increased protein solubility through modifying the pH of soaking medium; enhanced water uptake and thermal penetration by improving bean porosity (Liu and Bourne, 1995). These actions are influenced by the type, concentration and monovalent-to-divalent ratio of salt (de León, Elías and Bressani, 1992; Ávila *et al.*, 2015).

Further research is required to gain a better understanding of the fundamental mechanisms that lead to the development of HTC traits, and to elicit a viable, long-term solution to the problem.

2.3.4.2 Value chain constraints

Throughout Africa, Bambara groundnut is regarded by many as an important form of food security to be relied on when food is scarce (Lyimo, Berling and Sibuga, 2004; Mbosso *et al.*, 2020). Its tasty, nutritious properties are also recognised and valued by consumers, creating demand for the fresh seeds (Adzawla *et al.*, 2016). Additionally, various cultural and religious beliefs are associated with consumption of the crop (Berchie *et al.*, 2010; Forsythe *et al.*, 2015). Despite the importance attached to the crop, there remain several obstacles to promoting local utilisation of Bambara groundnut in the region. Inadequate (Katungwe, Mwangwela and Geresomo, 2015) and inconsistent supply (Adzawla *et al.*, 2016) are among the major barriers to wider consumption. Low market availability of the crop could be due to inefficient farming (Ani, Umeh and Ekwe, 2013), poor weather and soil (Berchie *et al.*, 2010), competition and displacement by cash-crop farming (Sidibé *et al.*, 2020), pests and diseases (Hillocks, Bennett and Mponda, 2012), lack of resources including access to high-quality seeds, land and storage, labour, capital and extension services (Adzawla *et al.*, 2016), and societal norms and beliefs that restrict cultivation, retail and consumption of Bambara groundnut (Forsythe *et al.*, 2015). Difficulty to process and prepare due to its HTC and hard-to-mill properties, are also reported to be one of the major concerns by consumers (Katungwe, Mwangwela and Geresomo, 2015; Mubaiwa *et al*., 2018). Besides, large-scale post-harvest processing is further limited by lack of capital, processing facilities and low product quality (Mbosso *et al.*, 2020). Other factors such as disliked by consumers and flatus-causing attribute also limit its local adoption and consumption (Adzawla *et al.*, 2016). Local demand and price may fluctuate over the year (Mbosso *et al.*, 2020), which might in turn discourage cultivation of the crop.

Seed selection criteria, production constraints and socio-economic challenges vary across regions. Therefore, local bodies may play a significant role in developing strategies and policies to effectively pinpoint and address the local challenges. Community biodiversity management

through provision of training and education, and enhanced seed access, has been proven to successfully improve adoption of Bambara groundnut in a few regions in Mali (Sidibé *et al.*, 2020). Tackling other value chain bottlenecks such as infrastructure, processing unit and market access, and raising awareness about the contribution of Bambara groundnut to agri-food systems, population health and community welfare, may prove crucial in encouraging more widespread local uptake of Bambara groundnut (Sidibé *et al.*, 2020). A complete value chain can help to ensure constant supply and reduced wastage, thereby preventing price fluctuation and enhancing utilisation.

The trade of Bambara groundnut has mostly been confined to adjacent villages (Mbosso *et al.*, 2020), suggesting that it is relatively unknown by the rest of the world. Promoting the use of Bambara groundnut at global levels through effective promotion strategies may drive to higher demand, which in turn could encourage cultivation and intervention policies by local and national bodies. Firstly, in order to increase its popularity worldwide, it would be sensible to first understand consumers' demand. Several studies indicated sensory attributes being the major determinant of consumer acceptance to novel Bambara groundnut products (Awobusuyi and Siwela, 2019; Hussin *et al.*, 2020). Certain pre-treatments e.g. dehulling, roasting, germination and fermentation have been shown to result in higher sensory scores (Nti, 2009; Awobusuyi and Siwela, 2019). Besides, product formulation is imperative in improving consumer acceptability. Correct formulations and substitution levels could sometimes yield products that are more desirable than conventional products (Awobusuyi and Siwela, 2019). Lack of familiarity with the ingredient could deter consumers, but this aspect could be remedied by incorporating traditional ingredients into product formulation (Katungwe, Mwangwela and Geresomo, 2015; Afolabi *et al.*, 2018). Additionally, pricing of the products should be taken into consideration to ensure they are affordable for most people. Lastly, marketing strategy of these products should be specialised for targeted consumer groups. Innovation, nutritional quality, and agroecological and health-

promoting features are among the factors motivating consumers to consume Bambara groundnut products (Hussin *et al.*, 2020; Yang *et al.*, 2020).

2.3.4.3 Food safety and allergenicity

It is essential that any undesirable, and potentially toxic, attributes of Bambara groundnut are addressed to improve utilisation. The presence of *Aspergillus flavus* and its aflatoxin has been detected in Bambara groundnut (Olagunju *et al.*, 2018). The study reported that uncontrolled fermentation resulted in the worst proliferation of the fungus, and that although roasting eliminated aflatoxin before storage, it could not suppress fungal growth during storage. This raised concerns about implications of quality control during post-harvest processing, food production and storage, especially in humid tropical areas and regions with limited resources. There is also a need for quality assurance by accredited food testing facilities to ensure compliance with food safety regulations before the product reaches consumers.

Another area of concern is the possible presence of allergenic proteins in Bambara groundnut (Astuti, Palupi and Zakaria, 2016). There is very little reported research specific to Bambara groundnut, but it is essential to determine the magnitude of the problem amongst the various cultivars and landraces if it is to be adopted and promoted widely as an alternative food or ingredient.

2.3.5 The role of Bambara groundnut in closing nutrient gap

The nutrition transition in Africa, characterised by a move away from the production and consumption of traditional staple foods, rich in starch and dietary fibre, to more palatable staples and cheap processed food, is one of the drivers of the decline in consumption of Bambara groundnut. Other typical impacts of the nutrition transition include a decrease in plant protein sources, such as legumes, and increased availability and consumption of energy-dense snack

foods, carbonated sweetened beverages, added sugar, fats and oils in food preparation (Steyn and Mchiza, 2014). Such changes in dietary pattern are propelled by economic and social development, urbanisation, and acculturation, and they affect people of all socioeconomic status. For the rich and those with increased disposable income, the shift to highly palatable refined carbohydrates and animal sourced protein is an aspirational goal. For others, the choice of food is dictated by circumstances. Trade liberalisation and increased availability and affordability of ultra-processed food, table sugar and cooking oil have elevated energy-dense, nutrient-free food to be the "food of necessity". These foods are affordable, palatable, and easy to prepare. Increases in fuel and electricity prices have hampered food preparation and forced households to resort to less nutritious processed food that requires little preparation.

Nutrition transition is associated with increases in non-communicable diseases (NCDs) in developing countries (Popkin, Adair and Ng, 2012). As a result, many of the developing countries, including the poorest, face the multiple burden of malnutrition. Hunger and under-nutrition, of especially energy and several micronutrient deficiencies, have not been successfully addressed in Africa while the epidemiologic transition is seen in the increased prevalence of obesity and other NCDs in many African countries (Bosu, 2015; Melaku *et al.*, 2019). The nutrition transition in developed countries presents well-identified features that help to predict consumption changes in Low and Middle-Income Countries (LMICs) as they go through socioeconomic changes. Taking lessons from intensive monoculture and heavy reliance on major crops in developed economies, as well as their impacts on agricultural sustainability and nutritional status, future policy development in LMICs should place emphasis on diversification of national food supplies, and the access to and affordability of diverse and quality diets.

Countries at different stages of structural transformation should employ different strategies to enhance the contribution of agriculture to diet quality and nutrition (Pingali, Ricketts

and Sahn, 2015). Pingali *et al*. (2015) suggested that low-productive agricultural systems should focus on yield enhancement, while maintaining production diversity and ensuring equitable conditions for working women. Diversification of food supplies has been shown to be negatively associated with indicators of the prevalence of undernutrition (Remans *et al.*, 2014). Climate and soil fertility are also critical to semi-subsistence agricultural production and human welfare in LMICs. As crops are grown for both household consumption and for income, there are multiple connections between these two factors and poverty and the health of the families that work the land. Bambara groundnut, being a climate-resilient and nitrogen-fixing legume, is an ideal candidate crop for diversifying local food production system while improving the nutritional status of the community.

The elevation of Bambara groundnut, from under-utilised to more mainstream crop, would theoretically be less challenging if industry led. By promoting its use at a commercial level, smallholder farmers, while producing enough to feed the family, can sell the surplus to industrial producers for monetary returns that can be then used to improve the quality of life and nutritional status of the family. To increase utilisation at the household level, it is important to address the HTC phenomenon. As evidenced by the health benefits detailed above, fortification or substitution of commercial products with Bambara groundnut can enhance the nutrient density of the products. There are numerous potential opportunities that deserve more research before they can be fully exploited. From a commercial point of view, demand for these products has to be created. Changing consumers' perceptions requires creative and effective marketing strategies, as is the case of quinoa (FAO, 2011). Sustainability and equity aspects should be considered and integrated into the entire value chain.

2.4 Conclusion

Despite being a minor crop, Bambara groundnut has the potential to play a role in combating food insecurity and malnutrition at household, national and global levels. It has high adaptability in various growing conditions and can produce reasonable yield under environmental stresses. Not only does Bambara groundnut fix nitrogen to improve soil health, it also increases crop yield when incorporated into intercropping system. These positive attributes address the supply chain gap by ensuring supply stability and sustainability in the face of climate variability and resource depletion. The genetic diversity across Bambara groundnut landraces allows crop improvement program to select for desirable agronomic, nutritional, and processing traits that are advantageous to improved food and nutritional security.

Knowledge of the detailed nutritional quality and physicochemical attributes of Bambara groundnut will enable the wider use and application of the crop in numerous food products, for instance as alternative flour, modified starch or protein isolate. The nutritional values, sensory attributes and functional characteristics of food products can be modified through appropriate processing techniques.

The hard-to-cook phenomenon, value chain bottlenecks and food safety remain the major constraints to increasing the widespread utilisation of Bambara groundnut. These obstacles could be addressed by different players across the value chain through increased collaborative and interdisciplinary approaches in order to realise the full potential of Bambara groundnut's contribution towards food and nutrition security.

Chapter 3: General materials and methods

3.1 Materials

Bambara groundnut samples were obtained from the Crops For the Future Research Centre (CFFRC, Malaysia). Twelve genotypes were selected, covering a diverse range of physical attributes, including seed coat colour and seed size (Table 1; Figure 4). These genotypes were grown in the same farming season in 2018 at the Field Research Centre, Broga, Malaysia, under the same growing conditions to minimise environmental influences on the parameters assessed. After harvest, the seeds were dried and deshelled, then sealed in polyethylene bags. The bags were stored together with drying beads in a seed barrel (Drystore®, Centor Thai, Thailand) at 20°C (RH 35 ± 5%). Prior to use, immature and damaged seeds and foreign material were removed from the samples.

The storage time of the seed materials used in this study (>2 year) was considerably longer than the <1 year "aged seeds" storage time of most studies (Koriyama *et al.*, 2017; Gwala *et al.*, 2020; Chen *et al.*, 2021). Hence, these samples can be considered as "aged seeds".

To ensure homogeneity of seed size, the seed samples used for the following studies were selected so that their weights fall within one standard deviation of their respective mean mass.

Table 1 List of Bambara groundnut genotypes used in this study.

No	Genotype	Testa Colour	Abbr.	Country of Origin
1	BCGC13107	Black	B_BCGC	Indonesia
2	Cikur2.1	Black	B_CIKU	Indonesia
3	IITA686	Black	B_IITA	Nigeria
4	IPB Bam 6	Black	B_IPBB	Indonesia
5	20Acc118CivB	Black	B_20AC	Ivory Coast
6	Cream Light Brown Eye	Cream	C_CLBE	Ghana
7	Exsokoto	Cream	C_EXSO	Nigeria
8	Kaaro	Cream	C_KARO	Nigeria
9	Nav4	Cream	C_NAV4	Ghana
10	Red11	Red	R_RED1	Ghana
11	Songkhla1	Red	R_SONG	Thailand
12	100SB16Anam-C	Brown	N_ANAM	Namibia

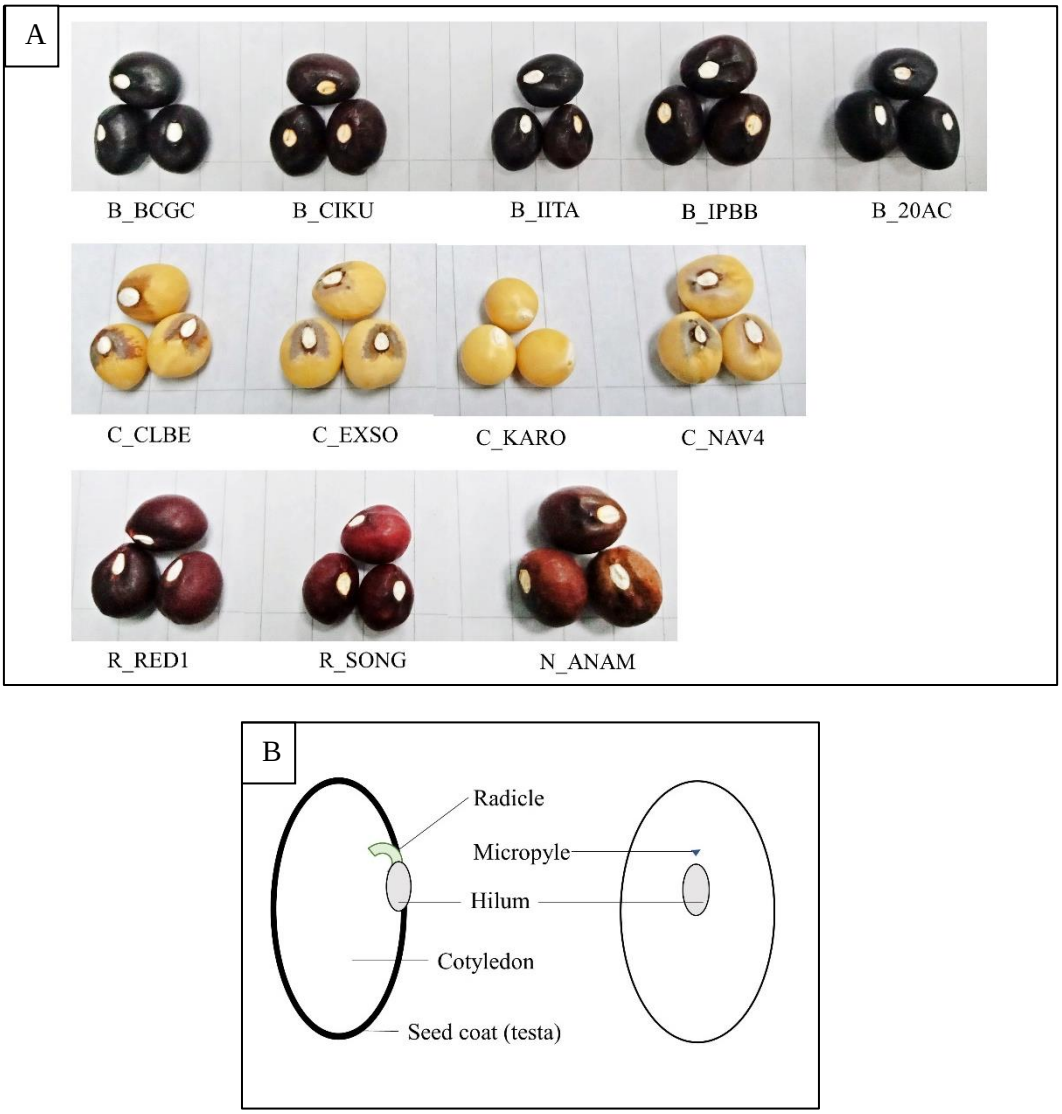

Figure 4 Twelve genotypes of Bambara groundnut used in this study (A) and the morphology of the seed (B).

3.2 General methods

3.2.1 Colourimeter

Colour was evaluated using a HunterLab MiniScan XE Plus Colourimeter (Hunter Associates Laboratory Inc., Virginia, USA). The colourimeter was set at illuminant D65 and standard observer 10° and calibrated against standard black and white tiles. Measurement was taken at the centre of the abaxial surface of seed (right angle to the cotyledon face). Results were expressed in the CIELAB colour scales: L* (0 = black; 100 = perfect white), a* (+a* = redness; -a* = greenness), and b* (+b* = yellowness; -b* = blueness).

3.2.2 Electrical conductivity (EC) meter

The EC was determined by a Field Scout Direct Soil EC Meter (Spectrum Technologies, USA) which has been calibrated with conductivity standard (2.76 mS cm^{-1}).

3.2.3 Refractometer

The soluble solid loss was determined according to Yeung *et al*. (2009). The soaking water was stirred to ensure homogeneity, then 0.5 mL of the broth was placed on the prism glass of a digital palette refractometer (PR-32α, Atago Co. Ltd., Japan). Soluble solid loss was calculated using the formula:

$$Solid\ loss\ (\%) = \frac{^{\circ}Brix \times Soaking\ water\ weight\ (g)}{Initial\ seed\ weight\ (g)} \times 100 \qquad (1)$$

3.2.4 pH meter

Prior to use, pH meter (Sartorius PB-10, Germany) was standardised with pH 4 and pH 7 buffer solutions.

3.2.5 Scanning electron microscopy (SEM)

Samples were mounted on stubs using double-sided carbon tape, sputter coated with platinum layer (Quorum Q150R Plus, Quorum Technologies Ltd., UK), and examined with a Field

Emission Scanning Electron Microscope (FEI Quanta 400F, FEI Company, USA) at an acceleration voltage of 20 kV.).

3.2.6 Differential scanning calorimetry (DSC)

Samples (5 mg) were accurately weighed into aluminium pans (Tzero, TA Instrument, USA) and deionised water was added at a ratio of 1:3 (w/v). The pans were hermetically sealed and left at room temperature for 24 h to equilibrate moisture. Thermal analysis was performed using DSC Q2000 (TA Instrument, USA). Nitrogen flow was set at 20 mL/min and samples were heated from 20 to 120°C at a rate of 5°C/min. Empty pan was used as reference.

3.3 Statistical analysis

All analyses were performed in triplicate unless otherwise stated. Data is presented as the means of replications ± standard deviation. Statistical analyses were performed using SPSS (version 26 and 28, IBM Corporation, USA).

Chapter 4: Physical, microstructural, hydration and cooking characteristics of twelve Bambara groundnut genotypes

4.1 Introduction

Grain quality is a determinant factor that governs the popularity and economic potential of Bambara groundnut. The physical properties, soaking characteristics, cooking quality and nutritional profile are among the major entities that determine the grain quality (Wang *et al.*, 2010; Wani, Sogi and Gill, 2013). Pulses are often sold in transparent packaging to display their quality. Therefore, their physical appearance, such as the seed size, shape, and colour, becomes an important quality attribute that influences the purchase decision by consumers. In addition, adequate information on the physical characteristics of legume seeds is of paramount importance to food processors in designing appropriate processing equipment for unit operations such as cleaning, sorting and drying (Baryeh and Mangope, 2003). Studies have also identified links between the physical properties, hydration characteristics and cooking time of different pulse seeds (Williams, Nakoul and Singh, 1983; Wani, Sogi and Gill, 2013; Miano *et al.*, 2018). Thus, the knowledge of physical traits could potentially be used to predict the water imbibition and cooking behaviour of Bambara groundnut, which, in turn, may be helpful for breeders in rapid screening of breeding lines.

In addition to the physical attributes, previous studies have suggested that grain microstructure could influence the rates of moisture diffusion and heat transfer in legume grains (Deshpande and Cheryan, 1986a; Miano, García and Augusto, 2015). Several structural features, namely the seed coat, hilum, and micropyle, have been associated with the water permeability of the seed (Sefa-Dedeh and Stanley, 1979; Tang, Sokhansanj and Sosulski, 1994). Although the relationship between anatomy microstructure of legume seeds and water imbibition

characteristics has long been recognised, there has been no report in the literature associating the variation in hydration behaviour of Bambara groundnut with the seed morphology and anatomy. Such study may provide complementary information on the role of these microstructural components and lead to a better understanding of the differences in the hydration behaviour.

Since Bambara groundnut is frequently dried post-harvest to prolong the storage period (Hillocks, Bennett and Mponda, 2012), soaking forms an integral part of subsequent processing of the dry seed, such as fermentation, cooking, and germination. Water imbibition is a physical process driven by the osmotic gradient between the seed and the soaking media (Miano and Augusto, 2018). During the hydration process, water diffuses into the legume grain, filling in the extracellular space and intracellular matrix, causing the starch granules and protein bodies to hydrate and swell, and the grain to expand (Wood, 2017; Chigwedere *et al.*, 2019). These changes facilitate starch gelatinisation, protein denaturation, polysaccharide solubilisation and cell separation during the subsequent thermal process, thereby shortening the cooking period. The hydration properties have been implicated to affect the cookability of cowpea (*Vigna unguiculata*) (Sefa-Dedeh, Stanley and Voisey, 1978), yellow field pea (*Pisum sativum*) (Wang, Daun and Malcolmson, 2003) and common bean (*Phaseolus vulgaris*) (Jones and Boulter, 1983; Deshpande and Cheryan, 1986b), whereby the seeds with higher water absorption rate tend to cook faster.

Previous studies on hydration characteristics of Bambara groundnut have successfully described the hydration kinetics using three mathematical models: the Peleg's model, the first-order model and the sigmoid model (Kaptso *et al.*, 2008; Jideani and Mpotokwana, 2009). However, the authors did not describe the storage length of the seed materials, and the factors associated with the variability in hydration behaviour were not investigated. It would therefore be of value to understand the hydration characteristics of different Bambara groundnut genotypes after extended storage, and to examine their potential role in affecting the cooking quality.

Cooking quality of pulses is determined by parameters such as the cooking time (CT) and the cooked texture and appearance. Extended cooking times for HTC seeds not only are time consuming and inconvenient, but also result in higher preparation costs due to increased expenditures on energy and clean water (Mubaiwa *et al.*, 2017). In regions where firewood is used as cooking fuel, gathering fuel wood is a labour-intensive task. Besides, clean water might not be readily accessible in the drought-prone Sub-Saharan regions where Bambara groundnut is processed and consumed. Therefore, the HTC attribute poses significant challenge to a wider consumption of pulses, especially for those from resource-scarce areas where legumes are valuable for food and nutritional security.

Much research has been devoted to address the hard-to-cook phenomenon of pulses. However, being an underutilised crop, information on the HTC phenomenon of Bambara groundnut is relatively scarce compared to that of the commonly consumed legumes such as common bean (*Phaseolus vulgaris*) (Kinyanjui *et al.*, 2015), soybean (*Glycine max)* (Koriyama *et al.*, 2017), chickpea (*Cicer arietinum*) (Reyes-Moreno *et al.*, 2000), and lentil (*Lens culinaris*) (Pirhayati, Soltanizadeh and Kadivar, 2011). The objectives of this study are to assess the inherent variability of the physical properties of Bambara groundnut as a function of genotype, and to correlate the physical traits with their respective microstructural, hydration and cooking characteristics to identify factors associated with HTC phenomenon.

4.2 Materials and methods

4.2.1 Seed Samples

Twelve genotypes of Bambara groundnut as described in Section 3.1 were used in this study. All samples were kept in the seed barrel until analysis.

4.2.2 Hundred seed weight

One hundred seeds of each genotype were randomly selected and weighed on an electronic balance (±0.01 g). Results were expressed as the mean of five replications (5 x 100 seeds).

4.2.3 Seed dimensions

For each genotype, the individual length (L), width (W), and thickness (T) (Figure 5) of 100 seeds were measured using a Vernier calliper with accuracy of 0.02 mm. Average of 100 measurements was reported.

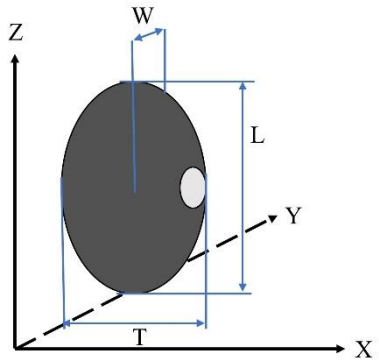

Figure 5 The three mutually perpendicular dimensions of the seed: length (L), width (W) and thickness (T).

Based on the three major dimensions, the following dimension parameters were calculated by assuming the seeds resembled an ellipsoid, according to Baryeh (2001):

a. Geometric mean diameter, D_g

$$D_g = (LWT)^{\frac{1}{3}} \quad (2)$$

b. Seed surface area, SA_S

$$SA_S = \pi \times {D_g}^2 \quad (3)$$

c. Sphericity, φ

$$\varphi = \frac{(LWT)^{\frac{1}{3}}}{L} \quad (4)$$

d. Calculated seed volume, V_C

$$V_c = \frac{\pi \times WT \times L^2}{6[2L - (WT)^{0.5}]} \quad (5)$$

The length-width (L/W) ratio and length-thickness (L/T) ratio were calculated as follows:

$$LW\ ratio = L/W \quad (6)$$

$$LT\ ratio = L/T \quad (7)$$

4.2.4 Hilum dimensions

The hilum length and width of 20 seeds were measured using a Vernier calliper (±0.02 mm). The length (L_H) and width (W_H) were defined as the major and minor diameter of the ellipse, respectively. The surface area of hilum (SA_H) was calculated according to:

$$SA_H = \pi \times \frac{L_H}{2} \times \frac{W_H}{2} \quad (8)$$

The specific hilum surface area (SSA_H) was defined as:

$$SSA_H = SA_H/SA_S \quad (9)$$

4.2.5 True density

The true density was determined using the liquid displacement method (Kaptso *et al.*, 2008). Distilled water (25 mL) was placed in a 50 mL graduated measuring cylinder. Twenty pre-weighed seeds were immersed in the water and the volume of water displaced was recorded. Due to the impermeability of Bambara groundnut seeds to water, it was assumed that there was no moisture uptake by the seeds (Kaptso *et al.*, 2008). The true density was calculated as:

$$\rho_t = \frac{weight\ of\ seeds\ (g)}{volume\ of\ displaced\ water\ (cm^3)} \quad (10)$$

4.2.6 Percentage of seed coat

Ten seeds were soaked in distilled water for 14 hours at room temperature. Before soaking, the dorsal side of the seeds was scored by a razor blade to aid water imbibition. After soaking, the seeds were blotted dry, and the seed coat was manually removed from the cotyledons. The two fractions were dried separately in an oven (Memmert, Germany) at 60°C until constant weight was achieved.

$$\%\ seed\ coat = \frac{Seed\ coat\ dry\ weight\ (g)}{Cotyledon\ dry\ weight\ (g) + Seed\ coat\ dry\ weight\ (g)} \times 100 \qquad (11)$$

4.2.7 Seed coat colour

The testa colour was evaluated according to Section 3.2.1. The mean value of 10 measurements was reported.

4.2.8 Seed microstructure

The morphology of seeds was examined in their raw, unfixed state as described in Section 3.2.5. Dry seeds were fractured transversely through the hilum with a scalpel blade to expose the cross-sections of seed coat, hilum and cotyledon. Whole seeds were used for the observation of the micropyle and the seed coat and hilum surfaces.

To determine the seed coat thickness, micrographs were captured on three points of seed coat cross-section: one on the dorsal side; two on the opposite abaxial side. The thickness was measured using ImageJ (National Institutes of Health, United States) based on the scale bar provided on the micrographs. Three measurements were taken from each micrograph. The average thickness (n=9) was reported.

4.2.9 Initial moisture content

The initial moisture content of seeds were determined by the AACC method for unground seeds (AACC, 1995). Approximately 5 g (accurately weighed to 0.1 mg) of whole seeds were oven dried at 103°C for 72 hours. Moisture content was calculated as:

$$Moisture\ content\ (\%\ dwb) = \frac{(W1 - W2)}{W1}\ \text{X}\ 100 \qquad (12)$$

where W1 = sample weight before drying (g) and W2 = sample weight after drying (g).

4.2.10 Rehydration kinetics

Ten seeds were weighed and soaked in distilled water at a ratio of 1:5 (w/w) at room temperature (25 ± 2°C). Sampling was carried out every 2 hours, whereby the seeds were removed from water, blotted dry with tissue paper, and weighed. The samples were immediately replaced into water to continue soaking. The duration of soaking was 48 hours for cream-coloured seeds and 60 hours for red-, brown-, and black-coloured seeds to ensure the seeds attained the equilibrium moisture content. The moisture content of the seeds (on dry weight basis, dwb) at each time interval was calculated as the percentage of sample mass increase per gram of dry seeds, taking into consideration the initial moisture content of seeds. Subsequently, changes in the moisture content of seeds, as a function of time, were used to plot a hydration kinetics curve.

Since Bambara groundnut seeds exhibited a sigmoidal hydration behaviour, the water absorption data were fitted to a sigmoidal model (Kaptso *et al.*, 2008):

$$M(t) = \frac{M_{eq}}{1 + exp[-k \cdot\ (t - \tau)]} \qquad (13)$$

where M(t) is the moisture content (% dwb) of seeds at time (t); M_{eq} is the equilibrium moisture content (% dwb); k ($hour^{-1}$) is the constant rate of rehydration; and τ (hour) is the time needed to attain half saturation (50%) of the seeds.

The coefficient of determination (R^2) and the mean relative deviation € were used to assess the goodness of fit of the model. The R^2 value was obtained during model fitting by SPSS, whereas E value was defined by the following equation (Wang, Daun and Malcolmson, 2003):

$$E(\%) = \frac{100}{N} \sum_{i=1}^{N} \frac{|m_e - m_p|}{m_e} \qquad (14)$$

where N is the number of experimental data; m_e and m_p are the experimental and predicted value, respectively. R^2 values close to 1 and E values below 10% indicate good fit of model.

4.2.11 Water entrance pathway into seeds

Genotype B_IITA was chosen for the study to determine the water entrance pathway due to seed availability. In the first treatment, the hilum of dry seeds (n=10) was covered with nail polish (Shine last & go!, essence, Luxembourg), as described by Miano *et al.* (2016). Nail polish was applied twice to ensure full sealing of the hilar region. Seeds were then soaked in distilled water at a ratio of 1:5 (w/w) at room temperature.

In the second treatment, seeds were pre-soaked for a specific time to achieve moisture content of 30% (dwb). The samples were kept in a sealed bag and placed in a refrigerator at 4˚C for 6 days to allow even moisture distribution throughout the seeds (Miano *et al.*, 2015). Thereafter, the hilum was covered with nail polish and the seeds were hydrated as described above. It has been suggested that seed coat permeability begins to decline at 25-30% moisture content (Hyde, 1954; Miano and Augusto, 2015). Thus, seed samples pre-soaked to 30% moisture content, but without their hilum covered, were used as control.

4.2.12 Electrical conductivity (EC), soluble solid loss (SL), pH and seed coat splitting

Hydration procedure was carried out as described in Section 4.2.10, except that ultrapure water (resistivity 18 MΩ) was used. Measurements were taken when the samples achieved 50% and 100% hydration levels. The EC, SL and pH of soaking solution was determined as described in Sections 3.2.2; 3.2.3 and 3.2.4, respectively.

Seed coat splitting was evaluated by counting the number of seeds with split seed coat. The results were expressed as the percent ratio of number of seeds with split seed coat to the total number of seeds.

4.2.13 Cooking time

Before cooking, seeds were pre-soaked to two hydration levels: 50% and 100% of their respective saturation moisture contents. The cooking times were referred to as CT-50S and CT-100S, respectively. After soaking, the seeds were drained, then cooked in distilled water (1:10 w/w) preheated to 100°C on a hotplate. During cooking (at 98 ± 2°C), the beaker was covered with a watch glass to reduce loss of water through evaporation. Samples (n=10) were withdrawn at specific intervals to test for softness by pressing between forefinger and thumb (Vindiola, Seib and Hoseney, 1986). The doneness was also determined by visually examining for the presence of whitish core. Cooking was considered complete when 80% of the sample disintegrated on pressing (Kinyanjui *et al.*, 2015) and the white core disappeared.

4.2.14 EC, SL, pH and seed coat splitting during cooking

Ten seeds of each genotype were soaked and cooked for the pre-determined cooking times (CT-50S and CT-100S). Subsequently, the seeds were removed, and the cooking broth was allowed to cool to room temperature. The EC, SL and pH of broth and seed coat splitting were determined as described in Sections 3.2.2; 3.2.3 and 3.2.4, respectively.

4.2.15 Water uptake during cooking

The cooked seeds were blotted dry and weighed. Changes in seed mass before and after cooking was recorded as water absorption during cooking. Changes in seed mass between cooked and dry seeds were taken as total water absorption.

4.2.16 Statistical analysis

A non-linear regression was conducted using SPSS (version 26, IBM Corporation, USA) for every soaking curve according to Equation 13. Pearson correlation was also performed using SPSS to correlate different parameters.

4.3 Results and discussion

4.3.1 Physical properties

4.3.1.1 Seed dimensions

The size of the seed can be described by its geometric dimensions. The principal dimensions (length (L), width (W) and thickness (T)) of Bambara groundnut varied considerably between and within genotypes (Figure 6). B_IPBB had the largest seed size in terms of L, W and T, with mean values of 13.87 mm, 10.65 mm, and 9.94 mm, respectively. In contrast, the lowest values of L, W, and T was observed in C_KARO (10.32 mm), R_SONG (8.84 mm), and C_NAV4 (8.48 mm), respectively. Generally, most of the L, W and T values observed in the present study were within the ranges (L=10.45-13.52 mm; W=8.81-10.49 mm; T=8.50-10.17 mm) reported for Bambara groundnut (Baryeh, 2001; Nti, 2009; Oyeyinka *et al.*, 2017).

The geometric mean diameter (D_g) was defined as the average of the three perpendicular dimensions of L, W and T, and henceforth was used as the equivalent diameter to define the surface area of seed (SA_S). Since the calculated D_g and SA_S are functions of L, W and T, the large-seeded genotypes also had high values of D_g and SA_S, and vice versa (Table 2). The D_g ranged from 9.40 (R_SONG) to 11.35 mm (B_IPBB), whilst the SA_S showed a considerable spread from 279 (R_SONG) to 407 mm^2 (B_IPBB). The genotypes exhibited wider ranges of D_g and SA_S compared to those reported in the literature (D_g = 9.59-10.5 mm; SA_S = 300-348 mm^2; Baryeh, 2001; Kaptso *et al.*, 2008; Mpotokwane *et al.*, 2008). This could be ascribed to the larger number of genotypes studied in the present study, indicating the benefits of using a wider range of genetically diverse seed materials. Such variations may provide a greater opportunity to select desirable traits during targeted breeding, thereby enhancing the breeding value of Bambara groundnut (Halimi *et al.*, 2019).

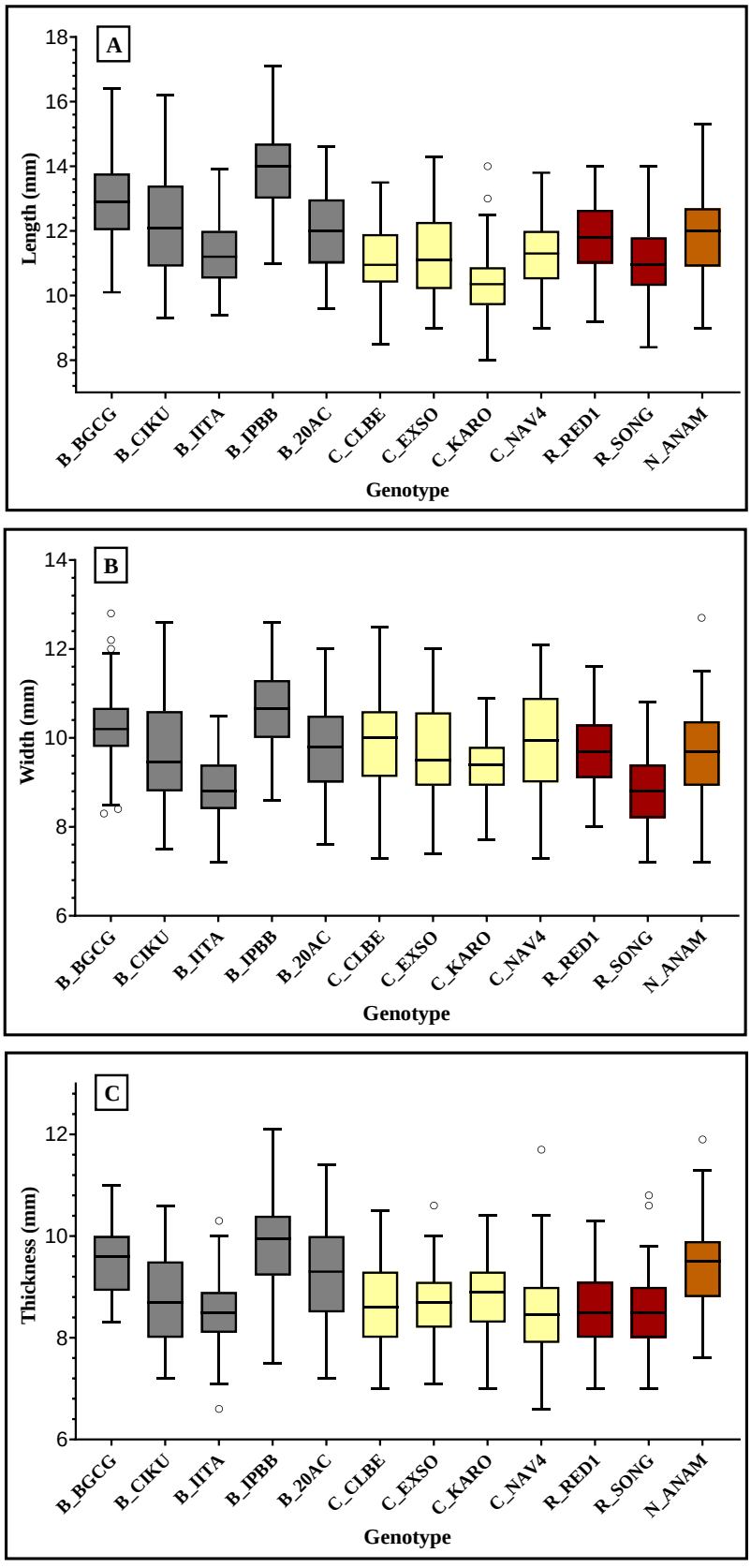

Figure 6 Boxplots of length (A), width (B), and thickness (C) of 12 Bambara groundnut genotypes. Boxes filled with grey, yellow, red, and brown represent black, cream, red and brown genotypes, respectively.

Knowing the shape of the seeds could help to predict the motion of seeds during processing and transport, and is important in the design of conveyors and hoppers (Mpotokwane *et al.*, 2008). The seed shape may also be related to water imbibition of pulses (Wood *et al.*, 2012), and this will be discussed in Section 4.3.5. Seed shape can be expressed in terms of sphericity, which is the relative measure of the similarity of the seeds to a perfect sphere. Value of 1 indicates a perfectly round sphere. C_KARO had the highest sphericity value (0.92), indicating that its shape was the closest to a sphere, whereas B_IPBB and B_CIKU showed the lowest sphericity value (0.82), indicating highest deviations from a spherical shape (Table 2). Nonetheless, with the sphericity value of >0.7, all genotypes can be considered to have a shape close to sphere, implying high tendency for the seeds to roll rather than to slide during processing (Oyeyinka *et al.*, 2017).

The length/width (L/W) and length/thickness (L/T) ratios also indicate the seed shape. The relative dimensions of Bambara groundnut seeds in terms of L/W and L/T ratios are illustrated in Figure 7. The value of L/W ratio was the highest for B_IPBB (1.31), indicating that it was the slenderest genotype, while the highest L/T ratio value of 1.40 were recorded for B_CIKU and B_IPBB, indicating that both genotypes had the flattest seed shape. In contrast, C_KARO had the most spherical seed shape with the lowest values of L/W ratio (1.11) and L/T ratio (1.17). These results correspond to the sphericity values discussed earlier. Generally, the L/W and L/T ratios of Bambara groundnut were lower than those of common bean (Deshpande, Sathe and Salunkhe, 1984), cowpea (Olapade *et al.*, 2002), lablab bean (*Lablab purpureus*) (Maass and Usongo, 2007), and lima bean (*Phaseolus lunatus*) (Giami, 2001), indicating a more spherical shape for Bambara groundnut. Overall, the cream-coloured seeds (plotted as ◊) had the lowest L/W ratio, followed by red- and brown-coloured seeds (plotted as Δ), whereas black-coloured seeds (plotted as o) had the largest L/W ratio. This trend was not observed in L/T ratio. The variations in seed size and shape across the twelve Bambara groundnut genotypes could be

attributed to genetic factors (Aliyu, Massawe and Mayes, 2016), but further studies are required to understand the genetic architecture of these traits.

All Bambara groundnut genotypes in this study had white, elliptically shaped hilum (Figure 4A), but their sizes varied between genotypes, as depicted in Table 2. Of all genotypes, B_IPBB had the largest hilum area, possibly due to its large seed size. Specific hilum area (SSA_H) is defined as the ratio of hilum area to the total seed surface area. Cream-coloured seeds (C_CLBE, C_EXSO, C_KARO and C_NAV4) generally had a higher specific hilum area than other genotypes. In contrast, R_RED1 had the lowest hilum area and specific hilum area. The detailed morphology of seed hilum and its role in water imbibition will be discussed in the following sections.

Table 2 Some geometric properties of 12 Bambara groundnut genotypes.

	Geometric mean diameter (mm)[1]	Sphericity[1]	Seed surface area (mm^2)[1]	Hilum area (mm^2)[2]	Specific hilum area[2]
B_BCGC	10.78 ± 0.74	0.84 ± 0.03	366.59 ± 50.09	6.96 ± 0.74	0.0190
B_CIKU	10.11 ± 1.04	0.82 ± 0.04	324.68 ± 67.30	8.63 ± 1.39	0.0266
B_IITA	9.48 ± 0.71	0.82 ± 0.04	283.70 ± 42.55	6.73 ± 1.21	0.0237
B_IPBB	11.35 ± 0.86	0.82 ± 0.04	407.30 ± 60.89	9.18 ± 1.18	0.0225
B_20AC	10.26 ± 1.00	0.85 ± 0.03	333.62 ± 64.90	7.83 ± 1.21	0.0235
C_CLBE	9.83 ± 0.89	0.88 ± 0.04	305.85 ± 55.30	8.04 ± 1.37	0.0263
C_EXSO	9.82 ± 0.88	0.87 ± 0.05	305.18 ± 54.99	8.33 ± 1.34	0.0273
C_KARO	9.44 ± 0.72	0.92 ± 0.04	281.87 ± 42.39	7.04 ± 1.41	0.0250
C_NAV4	9.81 ± 0.88	0.88 ± 0.05	304.67 ± 53.38	8.29 ± 2.09	0.0272
R_RED1	9.93 ± 0.75	0.84 ± 0.04	311.49 ± 47.23	5.86 ± 1.57	0.0188
R_SONG	9.40 ± 0.73	0.85 ± 0.04	279.41 ± 43.64	6.78 ± 1.23	0.0243
N_ANAM	10.25 ± 0.85	0.87 ± 0.04	332.14 ± 54.40	7.60 ± 1.95	0.0229

[1]Readings are means ± SD (n=100); [2]Readings are means ± SD (n=20)

Rows filled with grey, yellow, red, and brown colours represent black-, cream-, red- and brown-coloured genotypes, respectively.

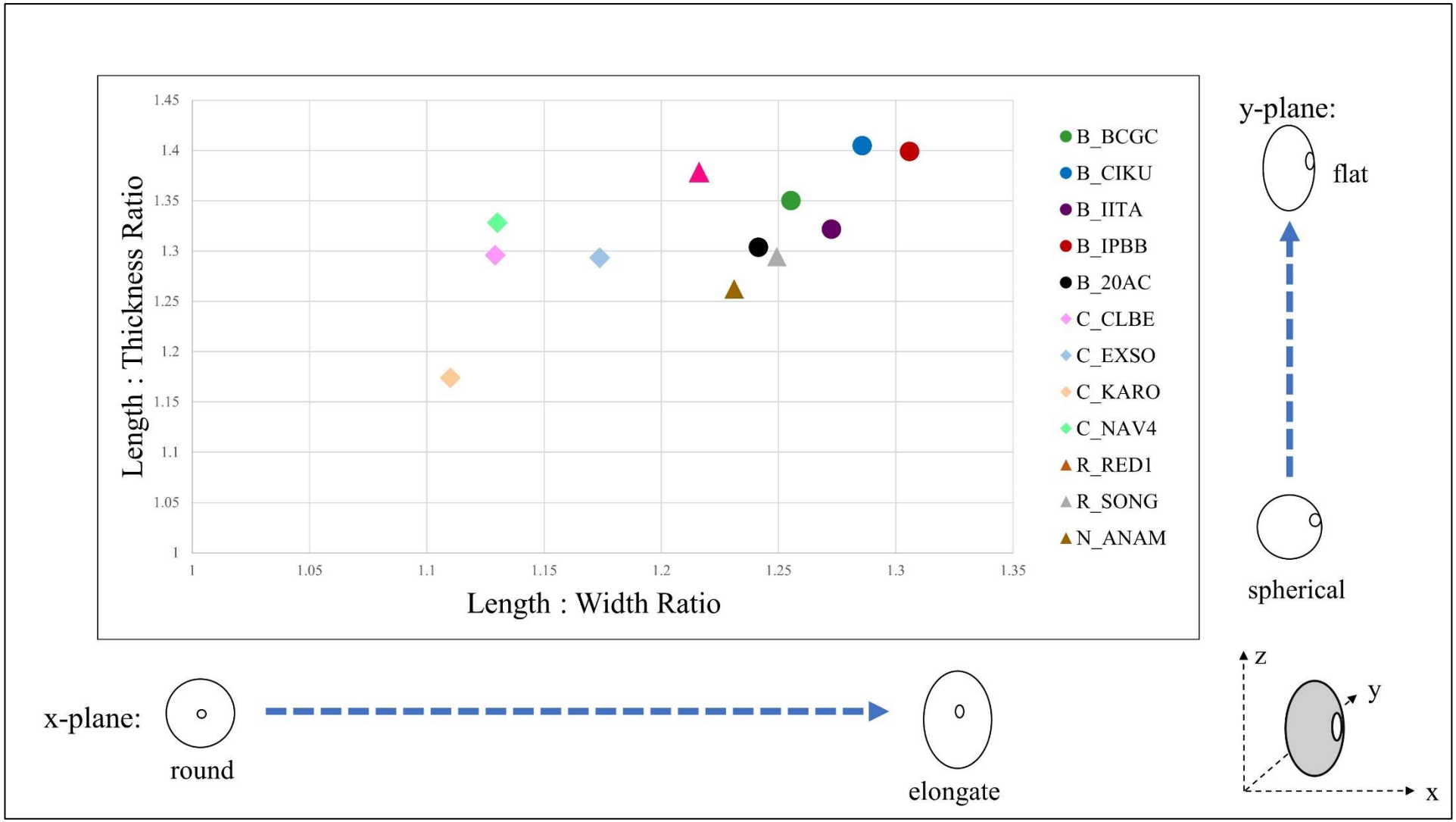

Figure 7 Relative dimensions of black- (●), cream- (◊), and red- and brown-coloured (Δ) genotypes of Bambara groundnut. The x-plane represents the ventral view of the seed (length: width ratio); the y-plane represents the lateral view of the seed (length: thickness ratio).

4.3.1.2 Mass, volume and density

Gravimetric properties, such as the weight per unit, are also measurable entities that can be used to describe seed size and to grade pulses. The 100-seed weight ranged almost two-fold, between 50.24 (R_SONG) and 92.30 g (B_IPBB) (Table 3). Despite the wide variation, the values were within the range documented for Bambara groundnut (50.30-93.30 g; Mpotokwane *et al.*, 2008; Nti, 2009). As with seed mass, the highest and lowest seed volume were recorded for B_IPBB (620.97 mm^3) and SONG (366.95 mm^3), respectively (Table 3). Strong positive correlations ($p<0.01$) were detected between D_g and 100-seed weight ($r=0.934$); D_g and seed volume ($r=0.988$); and seed volume and 100-seed weight ($r=0.951$), indicating that these parameters are good indicator in specifying the size of the seed.

The seed dimension, however, may not necessarily reflect the true density of seeds. This refers particularly to the cream-seeded C_KARO, which showed a relatively high mass despite a small volume. In contrast, B_CIKU exhibited a fairly low seed weight relative to its volume. Thus, C_KARO showed the highest true density at 1.42 g cm^{-3} and whereas B_CIKU showed the lowest at 1.07 g cm^{-3} (Table 3). Overall, the true densities were higher among the cream-coloured seeds (C_CLBE, C_EXSO, C_KARO, C_NAV4), similar to findings in other Bambara groundnut varieties (Kaptso *et al.*, 2008). A higher seed density may indicate a more compact cellular arrangement and a lower seed porosity, which might hinder water penetration into the cotyledon. It may also suggest the abundance of high molecular weight compounds in the seeds. Information of density can be useful particularly during removal of foreign materials and defective seeds during postharvest cleaning and sorting operations.

Table 3 Calculated seed volume (V_{calc}), 100-seed weight and true density of 12 Bambara groundnut genotypes.

Genotype	V_{calc} (mm^3)[1]	100-seed weight (g)[2]	True density (g cm^{-3})[3]
B_BCGC	541.12 ± 109.97	82.88 ± 2.56	1.22 ± 0.02
B_CIKU	445.87 ± 134.93	62.99 ± 1.73	1.07 ± 0.04
B_IITA	370.64 ± 83.85	58.65 ± 1.87	1.22 ± 0.04
B_IPBB	620.97 ± 137.23	92.30 ± 2.18	1.12 ± 0.04
B_20AC	481.54 ± 143.00	73.94 ± 1.89	1.24 ± 0.04
C_CLBE	437.63 ± 122.78	69.77 ± 1.59	1.32 ± 0.04
C_EXSO	428.92 ± 111.52	66.02 ± 1.89	1.24 ± 0.02
C_KARO	400.97 ± 85.52	60.94 ± 1.69	1.42 ± 0.01
C_NAV4	431.74 ± 120.03	63.23 ± 1.52	1.25 ± 0.04
R_RED1	426.54 ± 97.24	66.30 ± 1.72	1.23 ± 0.03
R_SONG	366.95 ± 86.88	50.24 ± 1.60	1.15 ± 0.05
N_ANAM	483.57 ± 121.45	65.58 ± 1.19	1.11 ± 0.03

[1]Readings are means ± SD (n=100); [2]Readings are means ± SD (n=5); [3]Readings are means ± SD (n=3)

Rows filled with grey, yellow, red, and brown colours represent black-, cream-, red- and brown-coloured genotypes, respectively.

4.3.1.3 Seed coat

The colour of the seed coat was assessed as CIELAB scales L* (lightness/darkness), a* (greenness/ redness) and b* (blueness/ yellowness) (Table 4). The L* and b* values were highest in the cream-coloured seeds, indicating that the seeds were lighter in colour with a higher degree of yellowness compared to other genotypes. In contrast, the black-coloured seeds had the lowest values for L*, a* and b*, indicating that they were the darkest. Traces of blue colour were detected in the black genotypes B_BCGC, B_IPBB and B_20AC, as indicated by the negative b* values. Unsurprisingly, the red- and brown-coloured seeds exhibited the highest a* values, which describes the degree of redness in the positive portion of the scale. Studies reported a decline in L value during storage period, and have associated the darkening of testa to the development of HTC defect in pulses (Reyes-Moreno *et al.*, 2000; Wacu *et al.*, 2015; Alves *et al.*, 2021). The darkening phenomenon during storage could be attributed to the oxidation of phenolic compounds (Nasar-Abbas *et al.*, 2008). Given that the samples used in this study had been stored for 2 years, it is possible that the seeds had undergone some extent of darkening.

The percentage of seed coat relative to seed weight was found to be the highest in the red-coloured genotypes, with R_RED1 and R_SONG recorded the values of 11.18% and 11.20% respectively (Table 4). Conversely, except for C_NAV4, the cream-coloured seeds had the lowest percentages compared to other genotypes. A negative correlation ($r=-0.777$, $p<0.01$) was found between percentage of seed coat and colour lightness (CIELAB L* value) of testa, indicating light-coloured genotypes tended to have thinner testae. Similar results were reported in other varieties of Bambara groundnut (Kaptso *et al.*, 2008), field pea (Black *et al.*, 1998), and lima bean (Giami, 2001).

Table 4 Seed coat characteristics of 12 Bambara groundnut genotypes.

	CIELAB parameters[1]			Percentage of seed coat (%)[2]	Thickness[3]	
Genotype	L*	a*	b*		Palisade layer (µm)	Total seed coat (µm)
B_BCGC	22.97 ± 1.80	3.74 ± 0.76	-0.69 ± 0.32	9.94 ± 0.14	87.73 ± 4.68	117.87 ± 4.47
B_CIKU	22.65 ± 1.28	3.63 ± 1.70	0.79 ± 0.75	10.72 ± 0.23	75.35 ± 5.61	113.08 ± 3.98
B_IITA	23.61 ± 2.77	6.27 ± 5.63	1.36 ± 2.87	10.50 ± 0.23	90.43 ± 7.92	120.00 ± 4.70
B_IPBB	22.07 ± 1.29	3.06 ± 1.86	-0.14 ± 0.73	10.10 ± 0.34	87.05 ± 4.27	126.84 ± 9.07
B_20AC	23.00 ± 0.99	2.16 ± 0.78	-0.62 ± 0.22	9.71 ± 0.57	88.26 ± 3.50	119.74 ± 2.87
C_CLBE	69.11 ± 3.79	6.47 ± 0.97	26.98 ± 3.21	8.77 ± 0.23	72.03 ± 13.33	105.57 ± 2.75
C_EXSO	70.39 ± 2.31	5.05 ± 0.56	27.40 ± 1.85	8.49 ± 0.69	72.86 ± 5.79	104.33 ± 5.87
C_KARO	75.78 ± 1.96	5.14 ± 0.56	27.09 ± 2.28	7.58 ± 0.63	70.62 ± 1.50	95.29 ± 1.26
C_NAV4	65.20 ± 2.85	7.70 ± 1.22	28.03 ± 2.48	9.90 ± 0.35	83.70 ± 14.35	108.10 ±10.62
R_RED1	26.77 ± 1.72	16.37 ± 2.07	6.76 ± 2.30	11.18 ± 0.29	96.66 ± 7.43	133.19 ± 7.33
R_SONG	27.77 ± 1.67	20.57 ± 4.08	7.70 ± 2.27	11.20 ± 0.40	79.75 ± 11.39	114.45 ± 5.04
N_ANAM	30.29 ± 4.21	15.12 ± 4.27	10.68 ± 5.84	9.59 ± 0.18	103.03 ± 9.41	131.92 ± 10.74

[1]Readings are means ± SD (n=10); [2]Readings are means ± SD (n=3); [3]Readings are means ± SD (n=9)

Rows filled with grey, yellow, red, and brown colours represent black-, cream-, red- and brown-coloured genotypes, respectively.

4.3.2 Soaking characteristics

4.3.2.1 Pathway of hydration

Based on visual observation, wrinkling and swelling of seed coat first appeared around the hilar region (Figure 8a), indicating that this was the site of initial water entry. The wrinkle and swelling then spread towards the dorsal side (the opposite side of hilum) (Figure 8b-8c), eventually disappeared once water had distributed evenly throughout the seed (Figure 8d). Using Magnetic Resonance Imaging autoradiography, Mandizvo and Odindo (2019) reported a similar hydration pathway in other Bambara groundnut landraces.

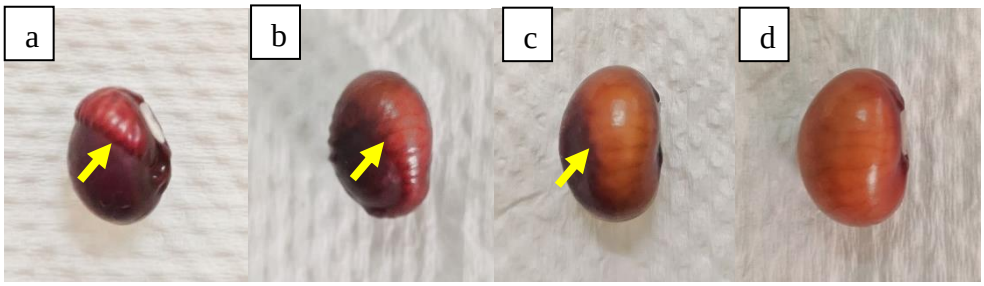

Figure 8 Hydration pathway of Bambara groundnut seed: (a) initial swelling of seed coat near the hilar region; (b) spread of swelling region towards the dorsal region; (c) the edge of swelling almost reaching the dorsal region; and (d) completely hydrated seed. Yellow arrows indicate the edge of swelling. Colour faded towards the end of soaking due to pigment leaching into soaking water.

It has been hypothesised that at low moisture contents, the seed hilum, serving as a hygroscopic valve, forms the major route of water migration into and out of the seed (Miano and Augusto, 2015). This hypothesis was tested by sealing the hilum before soaking (moisture content <10% dwb). As expected, the seeds became almost impervious to water and failed to imbibe water after 60 hours of soaking (Figure 9). The “hardshell” phenomenon thus indicates that at low moisture contents, seed coat was impermeable to water and the hilum was the principal water entry point for Bambara groundnut during initial soaking.

In another experiment, the seeds were pre-soaked to 30% (dwb) moisture content and allowed to equilibrate in the refrigerator for six days. In this case, the hydration curves for both covered and uncovered hilum showed a hyperbolic pattern, indicating rapid water uptake by the seeds (Figure 9). This shows that beyond a certain critical moisture content, the seed coat became permeable to water and, together with hilum, formed an integral water absorption system. Additionally, the seeds with waterproofed hilum exhibited a lower water uptake than the control seeds (hilum not sealed), indicating that the hilum still played an important role in water absorption at higher moisture levels. However, given the large surface area of seed coat as compared to that of hilum, it is plausible to suggest that water entry occurred primarily through the seed coat during this period. Hyde (1954) found that in clover and lupin seeds, 14% moisture content was the threshold for the testa to become totally impermeable, although its permeability began to decline at 25%. This trend was also observed by Miano and Augusto (2015) in Adzuki bean (*Vigna angularis*) and by Lush and Evans (1980) in wild cowpea (*Vigna unguiculata* (L.) subsp. *dekindtiana*).

When comparing the two control samples (hilum not blocked), it appears that the pre-soaked seeds hydrated faster than the unsoaked seeds. This was likely due to the testa of pre-soaked seeds being readily permeable to water after six days of moisture equilibration. This observation emphasises the importance of allowing uniform water distribution within the seed to moisten the entire testa, to fully establish the path for water entry through seed coat. The shift in water permeability of seed coat could be attributed to the plasticising effect of water on the hydrophilic polymers in testa, which led to the glass-rubber phase transition in the testa, consequently resulting in immediate moisture diffusion (Miano and Augusto, 2015).

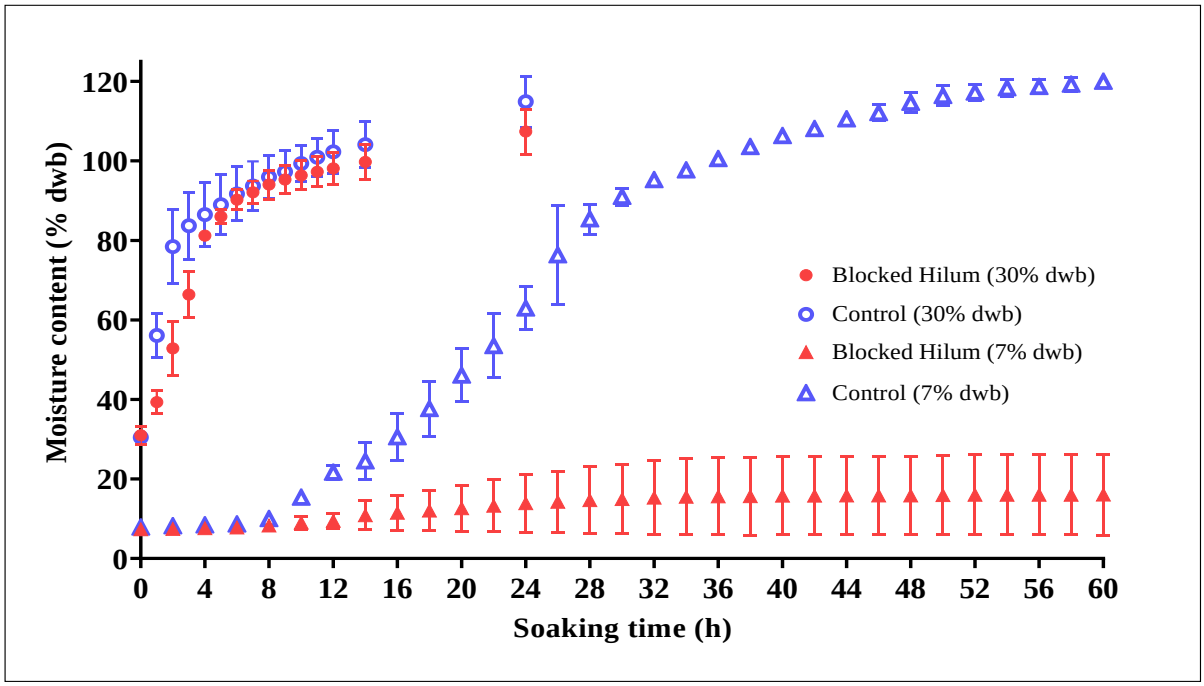

Figure 9 Hydration curve of B_IITA genotype with and without sealing of hilum at two initial moisture levels (7% dwb and 30% dwb). Markers are means of experimental values (n=3); vertical bars represent standard deviation.

4.3.2.2 Water absorption kinetics

The relationship between moisture content (on dry weight basis) of Bambara groundnut seeds and the duration of soaking is presented in Figure 10 (see Appendix 1 for individual plots with standard deviation). The hydration curves of all Bambara groundnut genotypes showed a sigmoidal shape, which was characterised by an initial lag phase, followed by a rapid water absorption phase until an inflexion point, after which the rate decreased as it approached a plateau phase at saturation. The initial moisture contents of all samples were low, ranging between 7.36 and 9.52% (dwb). As discussed earlier, at low moisture contents, the legume seed coat is impermeable to water and water enters the seed primarily through a partially closed hilum (Tang, Sokhansanj and Sosulski, 1994; Miano and Augusto, 2015). Therefore, the initial lag times in this study could be attributed to the adjustment periods for the hilum to open and allow water entrance. Although water diffused into the seeds at a relatively slow rate, the seed moisture content increased with soaking time. This led to widening of hilum's hygroscopic valve with a concomitant increase in seed coat permeability, thus resulting in increased water influx rate. As soaking continued, the water potential of soaking medium decreased due to leached solids from the seeds, whilst the water potential of the seeds increased (Del Valle, Stanley and Bourne, 1992). Consequently, the osmotic gradient decreased, and the water uptake rate started to decline at the inflexion point until it approached zero when the seed reached its maximum water capacity. A similar sigmoidal hydration pattern was also observed in Adzuki bean (Miano and Augusto, 2015), brown-seeded cowpea, and cream- and black-seeded Bambara groundnut (Kaptso *et al.*, 2008).

In the present study, all cream-seeded genotypes reached the plateau phase within 48 hours, whereas other genotypes required up to 60 hours to attain saturation. These extensive soaking periods to reach saturation moisture content were markedly longer than the 10-20 hours recorded in other varieties of Bambara groundnut (Kaptso *et al.*, 2008; Jideani and Mpotokwana,

2009). Water absorption characteristics of legume grains have been shown to be influenced by initial moisture content of seeds, microstructure and composition of seeds, soaking temperature, varietal differences and growing conditions, among other factors (Sefa-Dedeh and Stanley, 1979; Kaptso *et al.*, 2008; Miano and Augusto, 2015; Wang *et al.*, 2017). Since the initial moisture contents and soaking temperature of Bambara groundnut seeds in the current study were similar to those in studies by Kaptso *et al.* (2008) and Jideani and Mpotokwana (2009), the variation in hydration patterns between the present study and previous studies may be due to differences in cultivars or the use of aged seeds in this study, which could have influenced the microstructure and composition of seeds.

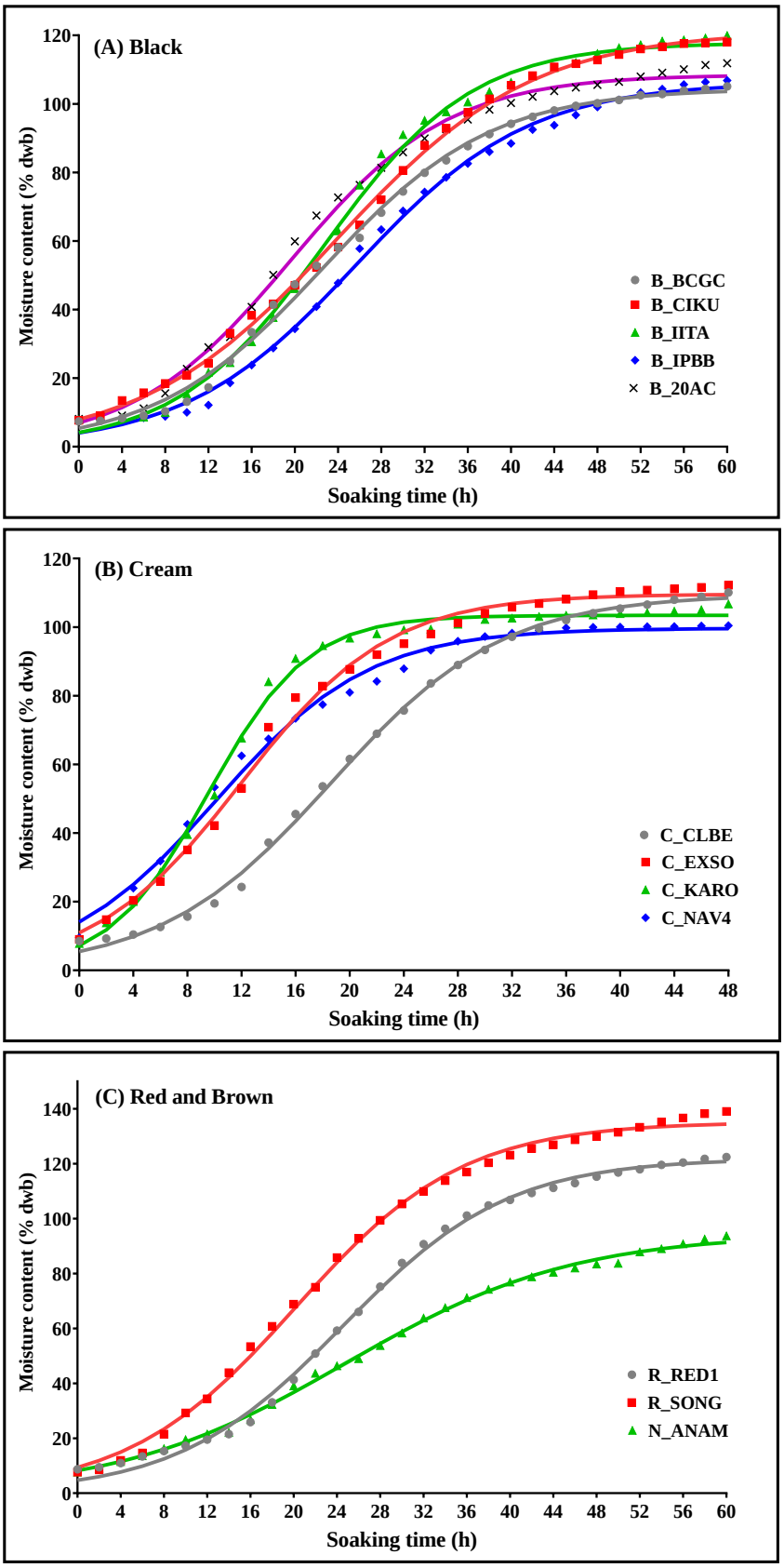

Figure 10 Hydration curves of (A) black-; (B) cream-; and (C) red- and brown-coloured genotypes, indicating moisture content as a function of soaking time. Markers are the averaged experimental data; solid lines are the predictive curve by model (Equation 13, Section 4.2.10).

4.3.2.3 Mathematical modelling

In order to compare the soaking characteristics of different genotypes, the experimental data was fitted to a sigmoidal model developed by Kaptso *et al.* (2008). Three parameters, namely the equilibrium moisture content (M_{eq}, % dwb), constant rate of hydration (k, $hour^{-1}$) and the time required to achieve half saturation (τ, hour), were estimated by the semi-empirical equation. For all genotypes, the R^2 and the E values were >0.99 and <10%, respectively, indicating good fit of the model to the experimental data (Table 5).

M_{eq} is defined as the maximum capacity of grain to absorb moisture. The M_{eq} values varied from 94.5-135.2 % (dwb) (Table 5), which were considerably higher than the values (85-88%) reported by Kaptso *et al.* (2008). Overall, both red-coloured genotypes, R_RED1 and R_SONG, were outstanding for having the highest M_{eq} among all genotypes, whereas the brown-coloured genotype (N_ANAM) showed the other extreme with the lowest M_{eq} value. The maximum water absorption capacity of seeds has important technological implications especially for canning operations. Legume grains with higher saturated moisture content tend to have a more substantial increase in seed size and weight after soaking, thus reducing the amount of seeds needed to fill each can, making them ideal for cost savings purpose (Wang, Daun and Malcolmson, 2003). A higher M_{eq} may also indicate a shorter cooking time. For example, it has been demonstrated in cowpea (Sefa-Dedeh, Stanley and Voisey, 1978) and chickpea (Gowen *et al.*, 2007) that increasing the moisture content up to the saturation point greatly softened the texture of the soaked seeds, implying a shorter cooking time required to soften the seeds.

The k value corresponds to the gradient of the curve, whilst the τ value is the time required to reach half saturation (the inflexion point of the curve) and is associated with the lag phase time. The values of k and τ were between 0.095-0.272 h^{-1} and 9.6-24.8 h, respectively. The k values were markedly lower than the range (0.31-0.63 h^{-1}) reported by Kaptso *et al.* (2008), whereas the τ values were substantially higher (2.1-5.7 h), indicating that the samples in this

study had a lower rate of water absorption and a longer lag phase. High hydration rate and short lag phase time are desirable for food preparation, as shorter soaking period implies less time and energy consumption. Notably, all four cream-coloured genotypes in this study had higher water absorption rates (higher k values; 0.158-0.272 h^{-1}) and shorter duration of lag phase (lower τ values; 9.6-18.7 h) than other genotypes ($k = 0.095\text{-}0.146\ h^{-1}$; τ = 19.6-25.7 h). N_ANAM exhibited the slowest hydration process, with its hydration rate almost 3 times slower than C_KARO's and its τ value was approximately 2.5 times longer than C_KARO's. The k and τ values of black-coloured genotypes were also systemically lower than cream- and red-coloured genotypes, indicating slower hydration. Previous studies on pea (Powell, Oliveira and Matthews, 1986), common bean (Del Valle, Stanley and Bourne, 1992) and other cultivars of Bambara groundnut (Kaptso *et al.*, 2008) also observed grains with light coloured seed coat to imbibe water more rapidly than their darker-coloured testa. Contradictory result, however, has been reported by Mandizvo and Odindo (2019), who found a lower imbibition rate in Bambara groundnut landrace with light-coloured seed coat. A larger screening study would be required to determine the adequacy of using testa colour as an indicator of hydration properties of Bambara groundnut.

Table 5 Estimated values of kinetic parameters of sigmoidal model (Equation 13) for 12 Bambara groundnut genotypes.

Genotype	M_{eq} (% dwb)	k (h^{-1})	τ (h)	R^2	E (%)
B_BCGC	104.618 ± 1.043	0.129 ± 0.004	22.655 ± 0.298	0.997	6.493
B_CIKU	121.320 ± 0.767	0.111 ± 0.002	23.922 ± 0.209	0.999	2.909
B_IITA	117.880 ± 0.896	0.146 ± 0.004	22.766 ± 0.238	0.997	6.438
B_IPBB	106.245 ± 0.954	0.126 ± 0.004	25.698 ± 0.278	0.997	7.366
B_20AC	108.545 ± 0.835	0.137 ± 0.004	19.602 ± 0.259	0.996	4.990
C_CLBE	109.501 ± 0.785	0.158 ± 0.004	18.655 ± 0.186	0.998	5.155
C_EXSO	109.609 ± 0.909	0.183 ±0.007	12.024 ± 0.236	0.995	3.356
C_KARO	103.429 ± 0.563	0.272 ± 0.010	9.558 ± 0.143	0.997	2.809
C_NAV4	99.664 ± 0.907	0.177 ± 0.008	10.194 ± 0.268	0.993	5.592
R_RED1	121.943 ± 0.964	0.131 ± 0.003	24.567 ± 0.247	0.998	7.513
R_SONG	135.179 ± 0.935	0.129 ± 0.003	20.152 ± 0.234	0.997	5.626
N_ANAM	94.549 ± 0.887	0.095 ± 0.002	24.773 ± 0.321	0.998	2.579

Readings are means ± standard errors (n=3).

Rows filled with grey, yellow, red, and brown colours represent black-, cream-, red- and brown-coloured genotypes, respectively.

4.3.2.4 Leaching losses

Assessment of the leaching losses provides information on the degree of seed deterioration and cell membrane degradation (Hentges, Weaver and Nielsen, 1991). It has been proposed that peroxidation of lipids in cell membrane during storage could lead to the loss of cell membrane integrity, subsequently causing increased solute leakage during soaking (Varriano-Martson and Jackson, 1981; Richardson and Stanley, 1991). This theory is supported by numerous studies which found increased electrolyte and solute leakage in HTC seeds during soaking (Plhak, Caldwell and Stanley, 1989; Berrios, Swanson and Cheong, 1999; Wacu *et al.*, 2015).

The electrical conductivity (EC), pH and amount of soluble solids loss (SL) of soaking liquid are shown in Figures 11A-11C. The EC and SL were used as a measure of the amount of electrolyte and soluble substances leached into soaking water, respectively. At half saturation point, the EC and SL values varied considerably with genotypes, ranging from 0.34-1.14 mS cm^{-1} and 0.42%-1.57%, respectively. Electrolyte and soluble solid leakage continued as soaking progressed. Thus, as the seeds reached saturation, the EC and SL of soaking water increased for all genotypes, ranging from 0.50-1.79 ms cm^{-1} and 1.13-2.70%, respectively. The EC was strongly correlated ($p<0.01$) with SL at both half saturation ($r=0.967$) and saturation ($r=0.935$) hydration levels, which is in agreement with the findings of previous studies (Liu *et al.*, 1992; Nasar-Abbas *et al.*, 2008). These results suggest that determining either one of EC or SL may be adequate when assessing soaking losses of Bambara groundnut in future study.

The pH of soaking water is an indicator of the amount of hydrogen ion leached from the seeds. Unlike the EC and SL, changes in pH of soaking water did not show a clear trend over soaking period. When the seeds attained 50% saturation, the pH of soaking water ranged from 5.23-5.83. However, when the grains reached the equilibrium moisture content, pH of soaking water of five genotypes increased, while that of the remaining genotypes decreased. Mandizvo and Odindo (2019) found that the pH of soaking water of Bambara groundnut showed a general

decreasing trend over 24 hours soaking period. As the seeds were soaked for a longer period in this study, there may also be possibility that fermentation had occurred during prolonged soaking, causing a decrease in the pH of the soaking medium.

4.3.2.5 Seed coat split

During soaking, the seed increased in size due to swelling of intracellular macromolecules and cell wall components, thus exerting pressure on the seed coat. When the seed expanded to a point where the seed coat could no longer withstand the pressure and maintain its integrity, the testa split open. Based on visual observation, the cracks were mostly perpendicular with respect to the long axis of the seed, first observed in the dorsal region. This suggested a greater expansion of seed in the direction of length during soaking, and that the presence of weak points could be more prevalent on the dorsal side. Ma *et al.* (2004) postulated that the dorsal side of soybean was more curved, thus causing the region to be structurally weak and consequently more prone to cracking.

When the seeds were partially soaked, the presence of transverse cracks was observed only in black-seeded B_CIKU and B_IITA (Figure 11D). However, when the seeds were soaked to their respective equilibrium moisture contents, the % split increased for most genotypes. Among all genotypes, the black-coloured genotypes exhibited higher incidence of seed coat splitting when the grains became fully hydrated. Conversely, despite having relatively thin testae, the cream genotypes showed none or low levels of splitting. It can be speculated that the seed coat of the black seeds is composed of fibrous components that give strength and rigidity to the testa, thereby causing resistance to water absorption and volume expansion during soaking. Such rigidity may also explain the lack of flexibility or extensibility of the testa, and therefore susceptibility to breaking when the cotyledons eventually swelled during soaking. Further histochemical study may provide clearer understanding of the morphology and composition of the seed coat to explain the observation.

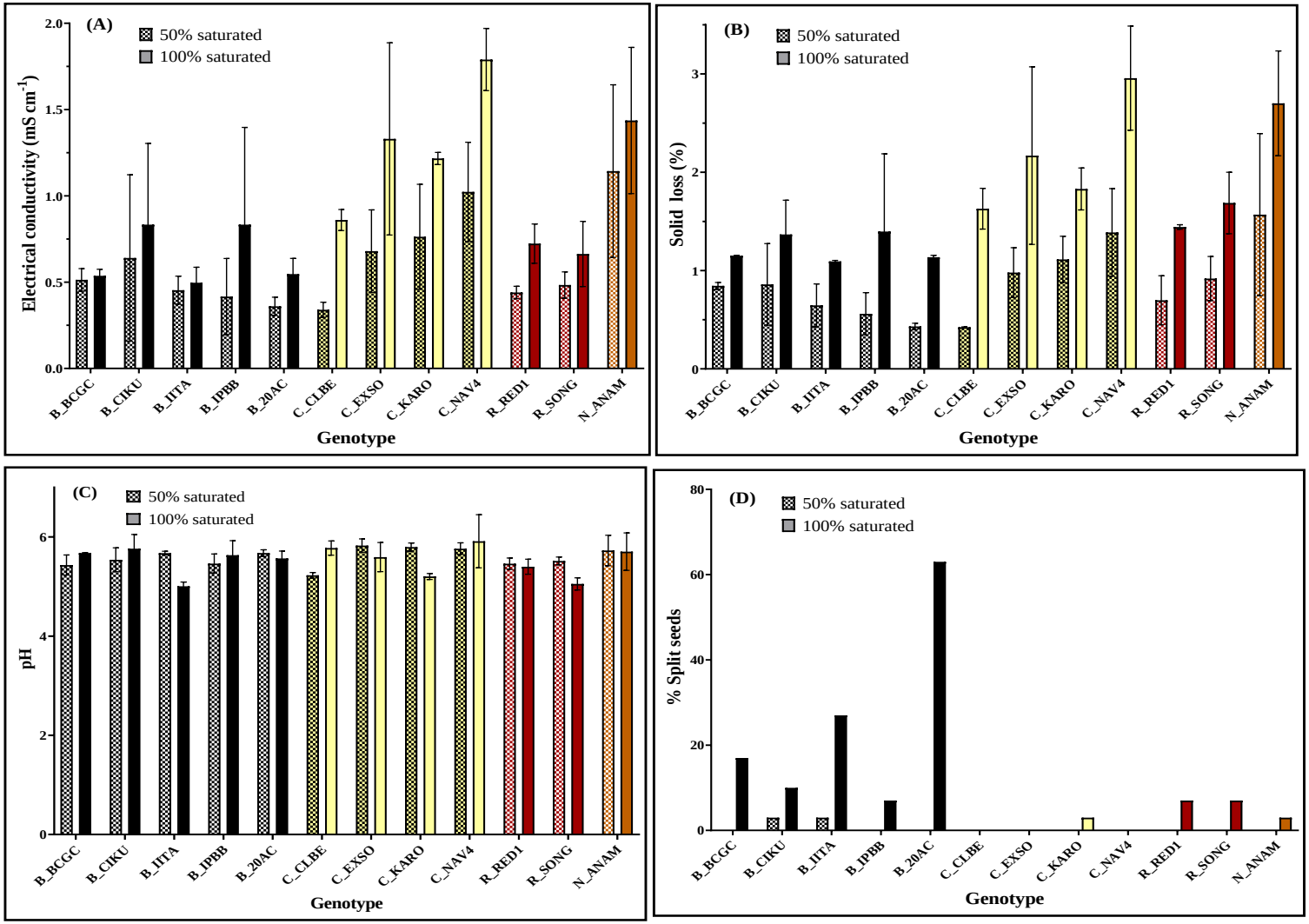

Figure 11 The EC (A), SL (B), and pH (C) of soaking liquid and percentage of split seeds (D) of Bambara groundnut genotypes when the seeds attained 50% (cross-pattern) and 100% (solid colour) hydration level. Vertical bars indicate standard deviation (n=3).

4.3.3 Seed microstructure

4.3.3.1 Microstructure of seed coat

There is consensus in the literature that seed coat is critical in influencing the permeability properties of legume grains (Sefa-Dedeh and Stanley, 1979; Agbo *et al.*, 1987; Ma *et al.*, 2004). Despite that the seeds appeared intact and smooth to naked eyes, minute cracks and holes were present on the testa of all genotypes when viewed under SEM (Figure 12). These pores and cracks could be naturally occurring or result from mechanical damage during processing such as harvesting, deshelling or transportation. Ma *et al.* (2004) also observed the presence of microscopic cracks on the surface of soybean seed coat, and attributed the formation of cracks to stresses caused by temperature or humidity fluctuations during processing and storage. The authors also suggested that the cracks were accountable for enhanced seed hydration. However, in this study, given that the dry seeds failed to imbibe water when hilum and micropyle was covered (Section 4.3.2.1), it is likely that these cracks were shallow and were not involved in the seed initial hydration.

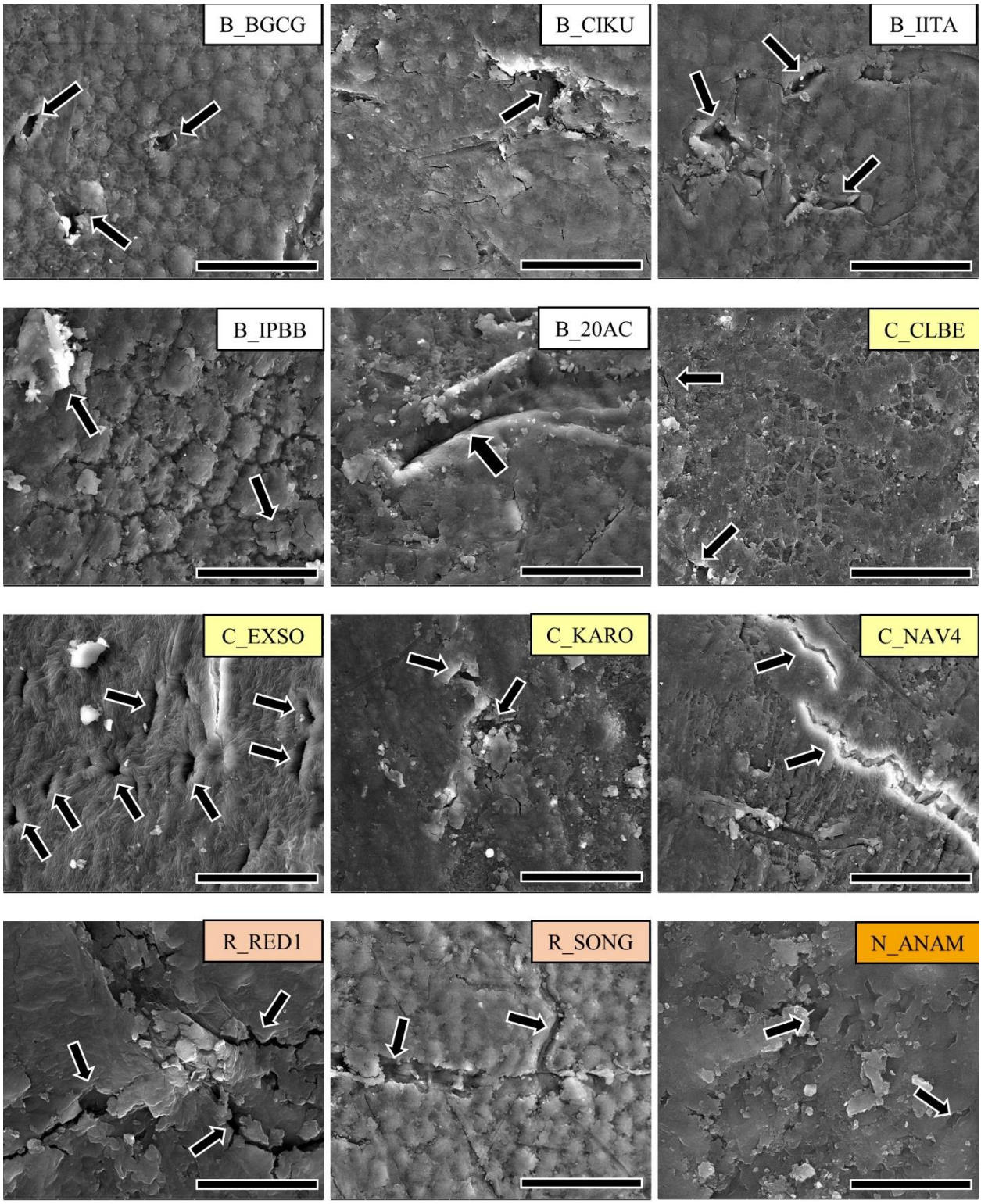

Figure 12 Enlarged view of the seed coat outer surface of 12 Bambara groundnut genotypes. Arrows indicate cracks and pits. Genotype names with white, yellow, red and brown labels represent black, cream, red and brown genotypes, respectively. Bar = 40 μm.

Figure 13 shows cross sections of the seed coat of Bambara groundnut. Although the general structure of seed coat did not show noticeable differences between the genotypes (Appendix 2a), the thickness varied considerably (95.92-133.19 μm) between genotypes (Table 4). The correlation between seed coat thickness and percentage of seed coat was significant ($r=0.673$, $p<0.05$). Similar to the seed coat percentage, the seed coat thickness was inversely correlated ($r=-0.809$, $p<0.01$) with testa colour lightness (CIELAB L* value). This finding was in accord with the observations of other authors working on Bambara groundnut seeds (Chibarabada, Modi and Mabhaudhi, 2014; Mandizvo and Odindo, 2019). One explanation is that consumer's preference for light-coloured seeds due to their sweet taste (lower tannin content) (Berchie *et al.*, 2010) and aversion for tough and fibrous seed coat may have driven farmers' selection of these traits during breeding, eventually leading to the co-occurrence of these characteristics over time.

The seed coat is the most external structure of the seed (Figure 4B). Its structure consists of multiple layers of cell, namely the cuticle, palisade, sub-epidermal (hourglass) and parenchyma (Smýkal *et al.*, 2014). Cuticle of legume seeds is a thin hydrophobic layer that covers the surface of testa. It has been proposed to cause water impermeability of seeds due to deposition of hydrophobic components such as waxes and hydrophobic proteins (Smýkal *et al.*, 2014). Below that is the palisade layer composed of tightly packed radially elongated macrosclereids, measuring about 70-103 μm in height (Table 4; Figure 13). This is the thickest layer in the Bambara groundnut seed coat and is strongly correlated ($r=0.914$, $p<0.01$) with testa thickness. Accumulation of polyphenolic compounds such as anthocyanins and proanthocyanidins in this layer contributes to the pigmentation of seed coat (Qutob *et al.*, 2008). Palisade layer is mechanically strong due to the thickened cell walls, providing strength and rigidity to the seed coat. As with the cuticle layer, palisade has been speculated to be related to seed coat impermeability (Ma *et al.*, 2004). It was found that cowpea varieties with thick and

compact palisade layer generally have a slow water imbibition rate, whilst those with thin and amorphous seed coats imbibe water at a faster rate (Sefa-Dedeh and Stanley, 1979).

Although indistinguishable in most of the micrographs in this study, other researchers identified a distinct hourglass layer lying underneath the palisade layer in many legume grains, including that of Bambara groundnut (Lush and Evans, 1980). The thin layer is composed of a single layer of dumbbell-shaped osteosclereids, and can be easily overlooked as a result of inappropriate sample preparation (Ma *et al.*, 2004). According to Swanson *et al*. (1985), chemical fixation or pre-soaking the seeds could help to reveal a more distinct hourglass layer. Because of its hourglass-shaped cells, this layer has large air spaces (Qutob *et al.*, 2008) which may be involved in moisture migration during seed hydration.

The amorphous and irregularly shaped parenchyma cells made up the inner-most layer of the testa, overlying the cotyledon (Figure 13). The parenchyma layer appeared to be thinner, less compact, and more porous than the palisade layer. It was theorised that this layer was more permeable to water movement than the palisade region and thus was responsible for water transport at the periphery of cotyledon (Smýkal *et al.*, 2014).

From an agronomic perspective, the seed coat functions as a protective barrier to the seed cotyledon, thus conferring resistance of seeds to damage, and against bacteria, fungal and pest infestation (Wang, Daun and Malcolmson, 2003; Maass and Usongo, 2007). In humid tropics, a thicker and impermeable seed coat is also imperative in preventing moisture penetration into the seeds, thereby promoting seed longevity (Lush and Evans, 1980). However, from a nutritional security viewpoint, legumes with thick seed coat could negatively affect the bioavailability of nutrients (Gwala *et al.*, 2020). This could be ascribed to the presence of antinutritional factors in the testa and the physical barrier imposed by seed coat thickened cell wall to impede digestion and absorption. Regarding food processing, thick seed coats have been associated with slower

water imbibition and increased cooking time of pulses (Wang, Daun and Malcolmson, 2003; Avola and Patanè, 2010; Miano, García and Augusto, 2015). Concerning sensory attributes, removal of highly pigmented seed coats, which are rich in tannins and fibres, has been shown to improve the sensory properties of food made from Bambara groundnut (Nti, 2009).

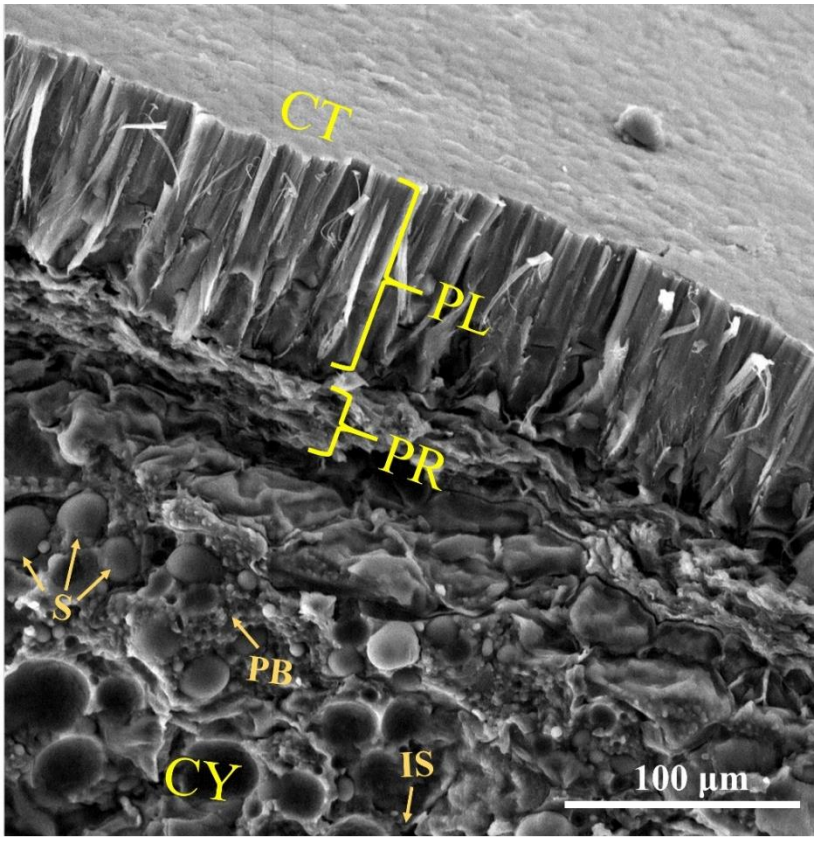

Figure 13 Cross-section of Bambara groundnut seed coat and cotyledon. The seed coat showing the cuticle (CT), palisade (PL) and parenchyma (PR) layers; the starch granules (S), protein bodies (PB) and intercellular space (IS) are visible in the cotyledon (CY) region.

4.3.3.2 Microstructure of cotyledons

The cotyledon constitutes a major portion of the seed in terms of volume and weight (Figure 4B). The microstructure of cotyledon has been associated with the HTC phenomenon in legume seeds (Varriano-Martson and Jackson, 1981). A transverse cross-section of Bambara groundnut cotyledon (Figure 13 & Figure 14) shows its morphology to be similar to that of other legume seeds (Sefa-Dedeh and Stanley, 1979; Wolf and Baker, 1980). The length and width of cotyledon cells ranged from 50-120 μm and 40-100 μm, respectively, similar to those of cowpea (Liu, Hung and Phillips, 1993), but larger than those of common bean (Agbo *et al.*, 1987). The cotyledon cells contained spherical- and elliptical-shaped starch granules, the size of which varied greatly between and within genotypes. The starch granules ranged from 10-50 μm, comparable to those (7-45 μm) obtained by Diedericks *et al.* (2020) from other Bambara groundnut varieties. The starch granules were surrounded by smaller-sized protein bodies, both of which act as storage reserves of essential nutrients required during seed germination.

Most genotypes exhibited various extents of intercellular cleavage, in which the cleavage plane occurred along the cell walls (Figure 14). Since fracturing did not occur across the cell wall, the intracellular contents were not visible, and observation was limited to the exterior surface of cell wall. This phenomenon might be due to tight packing of intracellular contents that affected the shearing action of the blade during fracturing (Liu, Hung and Phillips, 1993), or to weak intercellular adhesion which favoured cell separation through the middle lamella (Garcia, Lajolo and Swanson, 1993). Intercellular cleavage was prevalent in C_CLBE, C_EXSO and C_NAV4, and large concave depressions were clearly visible from the micrographs.

Although earlier studies (Varriano-Martson and Jackson, 1981; Garcia, Lajolo and Swanson, 1993) reported contraction and separation of cellular contents and plasmalemma from the cotyledon cell wall in some HTC common bean varieties, this was not observed in the present study. Nevertheless, the cotyledon cells of cream- and red-coloured genotypes appeared to be

more loosely packed and exhibited bigger volume and higher number of intercellular spaces. These voids may be involved in capillary flow of water throughout the cotyledon (Miano and Augusto, 2015), thereby enhancing the hydration rate of seed. Conversely, compact and strongly adhered cotyledon cells, often observed in HTC seeds, may partially account for impaired water penetration during soaking and cell separation during cooking (Cárabez-Trejo, Paredes-López and Reyes-Moreno, 1989; Berrios, Swanson and Cheong, 1997).

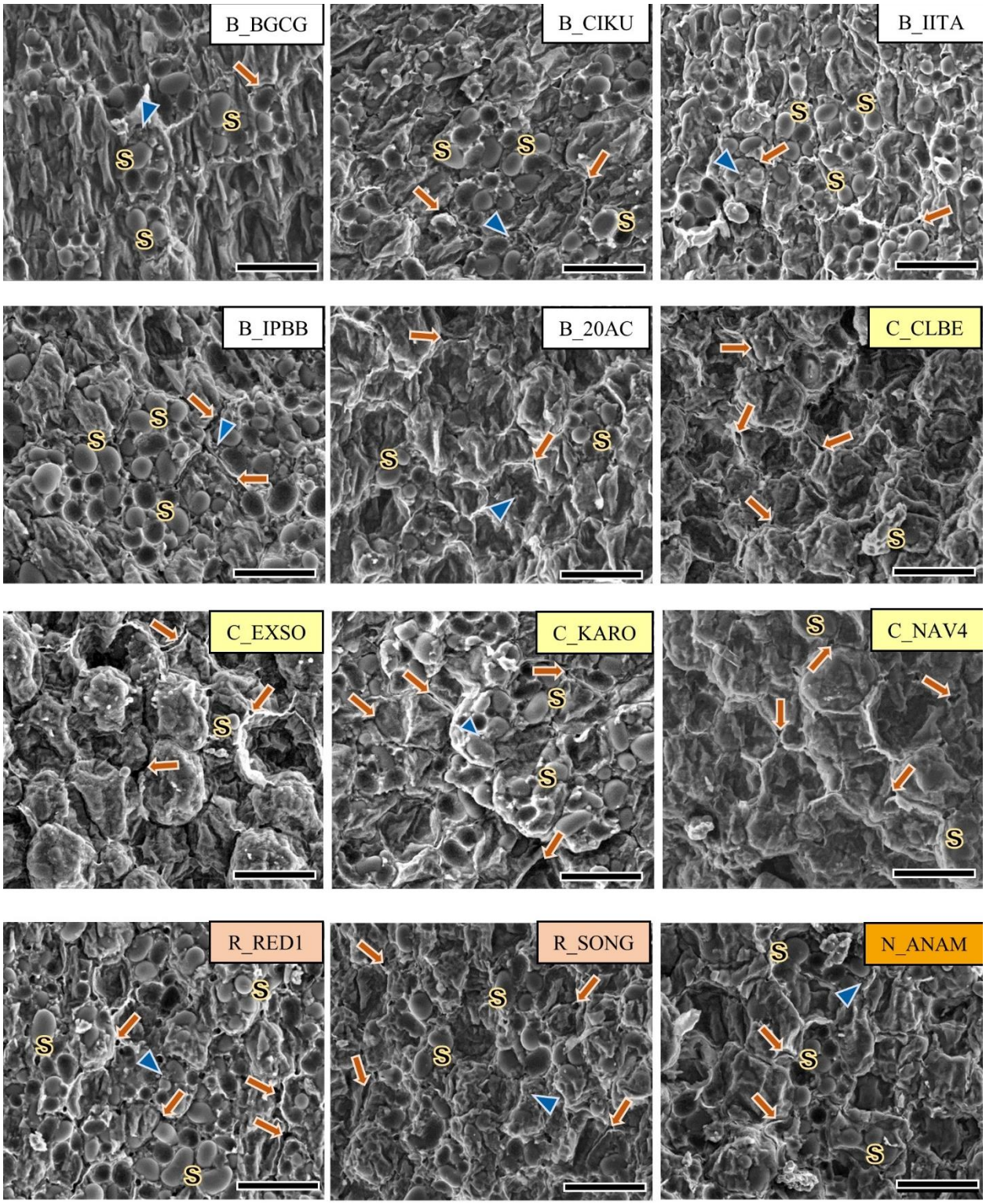

Figure 14 Cross-section of Bambara groundnut cotyledon cells. "S", blue triangles and, red arrows indicate examples of starch granules, protein bodies, and intercellular spaces, respectively. Genotype names with white, yellow, red and brown labels represent black, cream, red and brown genotypes, respectively. Bar = 100 μm.

4.3.3.3 Microstructure of hilum and micropyle

The hilum of Bambara groundnut (Figures 4B; 15) exhibited the anatomical structural characteristics of legume seeds (Lush and Evans, 1980; Agbo *et al.*, 1987). The hilum region, serving as the attachment point between the funiculus and the seed pod during seed maturation, was covered by a sponge-like surface layer. Below this were two layers of palisade-like cells. The outer layer, known as the counter-palisade, spanned across the hilar region, whereas the inner layer extended over the region, adjoining the palisade layer of seed coat (Figure 15A). The counter-palisade layer, containing a high concentration of hygroscopic compounds (e.g. galactomannans and pectins), is believed to be responsible for regulating the opening of hilar fissure (Lush and Evans, 1980). The double-layer palisade cells were interrupted along the centre by a narrow groove known as the hilar fissure or hilum groove, which acts as a valve that controls moisture migration into and out of the seed (Smýkal *et al.*, 2014). Winged bean (*Psophocarpus tetragonolobus*) with a slow hydration rate was also found to have a narrow hilar fissure (Deshpande and Cheryan, 1986a). Tracheid bar, the tear-drop shaped structure positioned below the hilar fissure, was surrounded by loosely arranged and irregularly structured vascular tissue. A close-up image (Figure 15B) reveals that the tracheid bar was composed of multiple layers of thin membranes with numerous long and narrow pits. This structure has been suggested to act as a screen filter in the water entry system of the seeds (Deshpande and Cheryan, 1986a). The tracheid bars of black- and brown-coloured genotypes appeared narrower and smaller in size than those of other genotypes (Appendix 2b). This structural feature may have partially contributed to their long lag phase and slow hydration. Tracheid bar could not be identified in genotype R_RED1 and it is not clear whether this is due to the absence of the structure in this genotype or inappropriate sample preparation method.

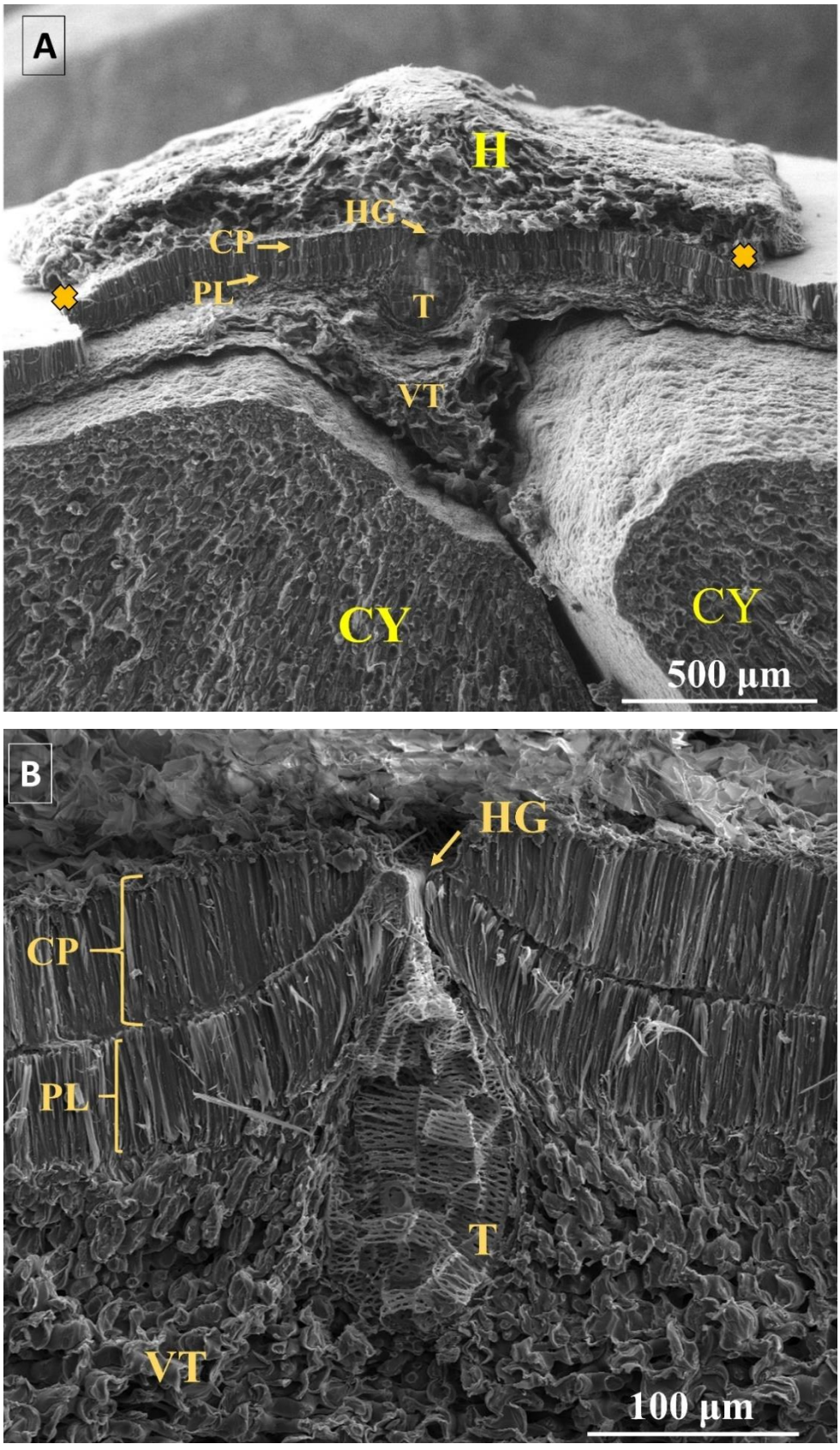

Figure 15 Cross-section of hilar (H) region of Bambara groundnut at 150x (Fig 15A) and 300x (Fig 15B) magnifications, exhibiting the hilar groove (HG), counter-palisade (CP) and palisade (PL) layers, tracheid bar (T), vascular tissue (VT) and cotyledon (CY). The crosses (x) in Figure 15A show the end of counter-palisade layer.

The microstructure of the external surface of hilum was also examined (Figure 16). The hilum surface of all genotypes showed randomly organised, mesh-like porous structure, similar to that observed in other *P. vulgaris* varieties such as black bean (Berrios, Swanson and Cheong, 1997) and carioca bean (Miano *et al.*, 2018). The network structure was interconnected by membrane-like structure.

In addition to hilum, the micropyle, a microscopic opening below the hilum (Figure 4B), has been proposed to be one of the major water entry points for some legume seeds (Sefa-Dedeh and Stanley, 1979; Deshpande and Cheryan, 1986a). The micrographs revealed that all genotypes had a "Y"-shaped, occluded micropyle (Figure 17). Such occluded micropyle has been suggested to be related to poor imbibition properties in cowpea (Sefa-Dedeh and Stanley, 1979) and common bean (Agbo *et al.*, 1987).

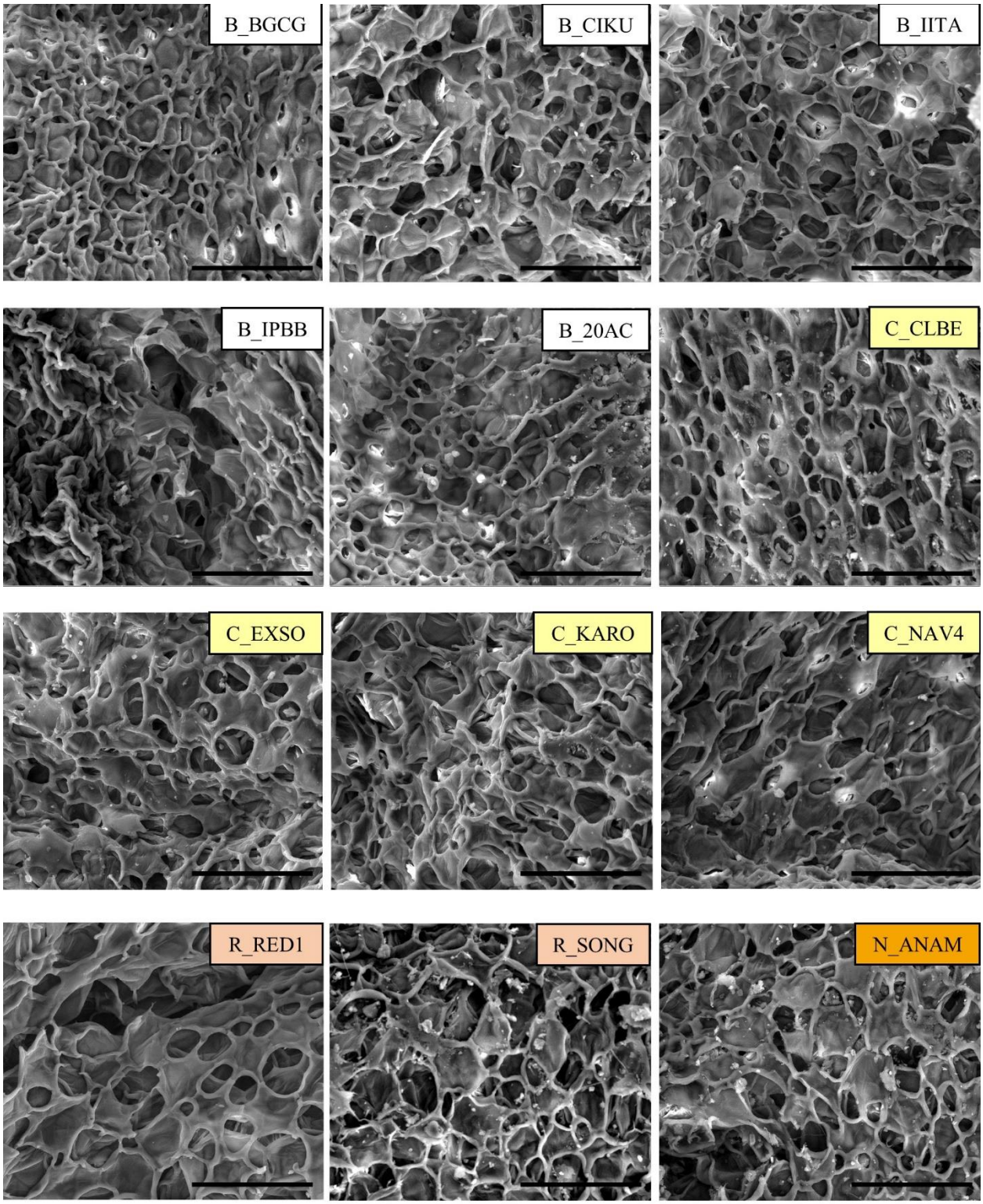

Figure 16 External structure of Bambara groundnut hilum. Genotype names with white, yellow, red and brown labels represent black, cream, red and brown genotypes, respectively. Bar = 100 μm.

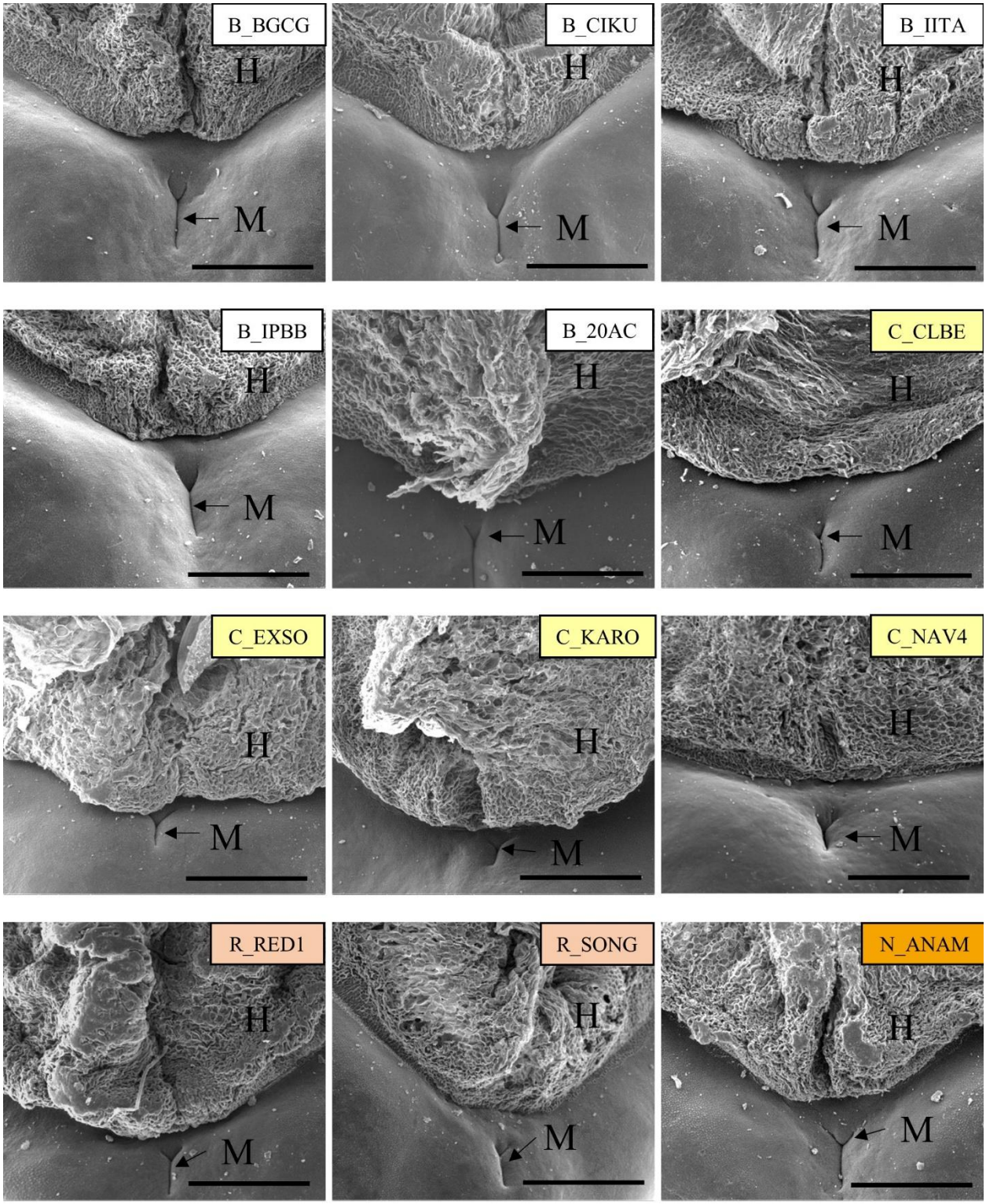

Figure 17 Enlarged view of Bambara groundnut hilum (H) and micropyle (M). Genotype names with white, yellow, red and brown labels represent black, cream, red and brown genotypes, respectively. Bar = 400 µm.

4.3.4 Cooking characteristics

4.3.4.1 Cooking time

The cooking times (CTs) established in this study were measured by subjective methods, that is, by finger-pressing method and visual observation on the disappearance of white core. Hence, the values obtained were of approximation and may be subject to high variability depending on the pressure applied during pressing. The CTs, nevertheless, were used to compare the relative cookability among the genotypes. The results indicate that the cookability of partially and fully hydrated Bambara groundnut varied greatly between genotypes, with the CTs ranging from 70-208 mins and 38-120 mins, respectively (Figure 18A). Of all genotypes, C_KARO was the fastest-cooking genotype at both 50% and 100 % hydration levels, whereas N_ANAM was the most time-consuming to cook, clearly exhibiting the HTC trait. The CT of seeds pre-soaked to 50% saturation (CT-50S) was strongly correlated ($r=0.810$, $p<0.01$) with the CT of seeds pre-soaked to their respective saturation moisture content (CT-100S), suggesting that cooking partially soaked seeds could be a rapid method to predict the cookability of Bambara groundnut in future studies. Overall, all genotypes exhibited shorter CTs as seed moisture levels increased. This observation parallels those documented in lima bean (*Phaseolus lunatus* L.) (Giami, 2001), black gram (*Phaseolus mungoo* L.) (Wani, Sogi and Gill, 2013) and red kidney bean (*Phaseolus vulgaris* L.) (Jian *et al.*, 2017). These studies also recorded a lower variability in CTs between genotypes as hydration levels increased, as was noted in the present study.

In addition to genotypic variation, seed-to-seed variation within each genotype was observed during the trial. Gwala *et al.* (2020), who used a texture analyser to measure the hardness of individual cooked Bambara groundnut seeds, also reported wide intrapopulation variability. Additionally, aged seeds tend to exhibit even greater seed-to-seed variation in their CTs compared to fresh seeds (Jackson and Varriano-Martson, 1981). This may also explain the

within-sample variability in the Bambara groundnut cooking behaviour, since the seed materials used had been stored for two years prior to the study.

The cell membrane deterioration hypothesis postulates that ageing-induced hardening of seeds is accompanied by increased leaching losses during soaking due to cell membrane degradation. In this study, CT of fully hydrated seeds was positively correlated ($p<0.05$) with the amounts of leached electrolytes ($r=0.583$) and soluble solids ($r=0.596$) during soaking. These results were in accord with those previously reported for other pulses (Hincks, Mccannel and Stanley, 1987; Hentges, Weaver and Nielsen, 1991; Richardson and Stanley, 1991) and corroborated the membrane deterioration hypothesis (see Section 1.3).

Regardless of the hydration level before cooking, the seeds continued to absorb water during cooking. The amount of water uptake during cooking showed genotypic difference (Figure 18B). All fully hydrated seeds exhibited limited water uptake during cooking but yielded greater cooked weights than partially soaked seeds (Figure 18C) due to higher water absorption during soaking. High cooked weight is desirable for commercial canning, as fewer seeds are required to obtain the same product yield. Furthermore, fully hydrating the seeds before cooking led to uniform textural changes of individual seeds during cooking, which is also a desirable attribute for canned beans. Physical observation during the soaking and cooking trial found a higher incidence of uneven cooking in partially soaked seeds, where the periphery of the cotyledons was overcooked (mushy) while the core remained hard and uncooked, indicating that the softening rate of hydrated region was faster than the rate of water penetration to the unhydrated (centre) region of the cotyledon. Al-Nouri and Siddiqi (1982) reported a similar observation in broad bean (*Vicia faba*).

Although pre-soaking the seeds to a higher hydration level shortened the CT, increased product yield and promoted uniformity of textural changes, such lengthy soaking period at room

temperature for Bambara groundnut may result in microorganism proliferation (Gowen *et al.*, 2007). This could pose a food safety challenge, especially for domestic cooking in tropical climates with limited technology and resources to control soaking temperature. Furthermore, from industry processing point of view, grains with extensive hydration period would increase processing time and, therefore, additional storage equipment and processing space for soaking process. On this basis, cream-seeded genotypes might be advantageous as they achieved saturation within a shorter duration of soaking.

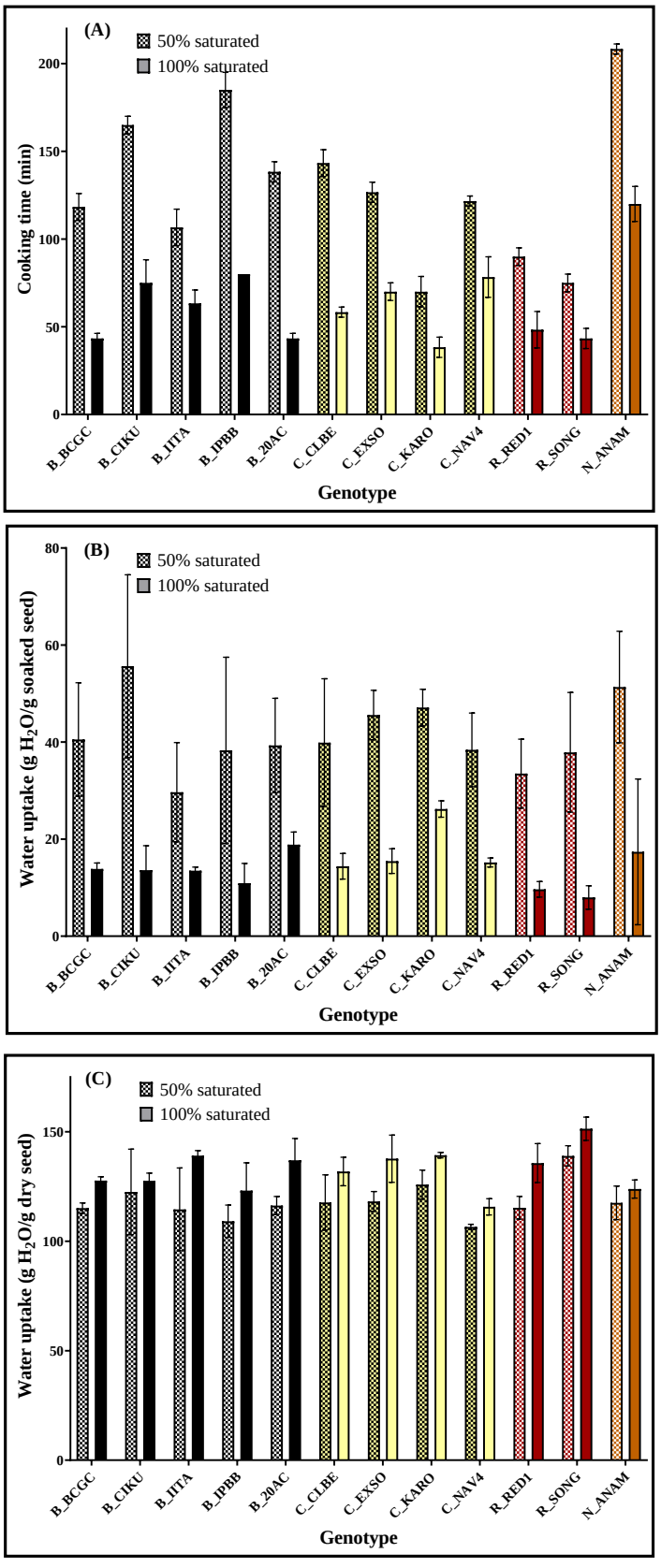

Figure 18 The cooking time (A), water uptake during cooking (B), and total water uptake (C) of Bambara groundnut genotypes when the seeds were pre-soaked to 50% (cross-pattern) and 100% (solid colour) saturation. Vertical bars indicate standard deviation (n=3).

4.3.4.2 Cooking losses

Boiling can cause considerable leaching of both nutrients (e.g., protein, lipids, and calcium) and ANFs (e.g., tannins) in Bambara groundnut (Ndidi *et al*., 2014). As with soaking losses, there was a large variation in cooking losses between the Bambara groundnut genotypes (Figure 19A-19C). Overall, the leaching during cooking was greater than that during soaking. The cream-seeded C_NAV4 was outstanding for its high leakage during soaking (Figure 11A), but relatively low leakage during cooking, possibly because the solutes had been leached out during soaking. The EC of cooking water for seeds soaked to 50% and 100% saturation level before cooking ranged from 1.30-2.04 mS cm^{-1}, and 1.25-1.89 mS cm^{-1}, respectively. Except for genotypes C_CLBE, R_RED1, and R_SONG, the fully hydrated seeds had lower electrolyte leakage during cooking than the partially hydrated seeds. This could be attributed to excessive leaching of electrolytes during extended soaking period, thus causing lower leakage during cooking.

Soluble solid loss followed a similar trend to the electrolyte leakage, with readings ranging from 5.73-9.76% and 5.91-7.60% for partially and fully hydrated seeds, respectively (Figure 19B). A strong positive correlation was found between the EC and SL in cooking water of partially and fully hydrated seeds ($r=0.846$ and 0.946, respectively; $p<0.01$). This again indicates that future study may determine cooking losses by evaluating either one of EC or SL.

Cooking water generally had higher pH levels compared to soaking water (Figure 19C), suggesting higher amounts of anions leaching during cooking. Similar findings were reported by Liu *et al.* (1993) in cowpea.

4.3.4.2 Seed coat splitting

Factors such as initial cracks on seed coat surface, mechanical stress due to rapid influx of water, and seed coat fragility or rigidity due to its composition, have been suggested to result in

seed coat splitting during cooking (Del Valle, Stanley and Bourne, 1992). Visually, as with splitting during soaking process, dorsal region was the primary site of splitting, and the splits were mostly perpendicular with respect to the long axis of the seed. Overall, incidence of seed coat splitting increased towards the end of cooking. Genotypes B_20AC and B_BCGC showed the highest percentage of split at both hydration levels (Figure 19). Increasing hydration level before cooking resulted in a greater % split during cooking for all genotypes. This trend was noted by Van Buren *et al.* (1986), who established a linear relationship (R^2=0.63) between % split during cooking and pre-soaked weight of kidney bean.

Since the seed coat acts as a physical barrier to retain solids within the seed, splitting was expected to result in higher solid loss. However, % split in this study was not correlated ($p>0.05$) with cooking losses, contrary to that seen in cowpea (Taiwo, Akanbi and Ajibola, 1998; Yeung *et al.*, 2009). This implies that seed coat may not be the key factor affecting the cooking losses of Bambara groundnut. Other factors, such as the solubility or binding strength of the components that leach out, may be involved in the leaching mechanism (Gwala *et al.*, 2020).

Being the fastest-cooking genotype with the shortest soaking time, C_KARO would be a good candidate for domestic cooking. However, since the C_KARO seeds were prone to split at the end of cooking, it is not ideal for canning operation where maintaining integrity of cooked grain is an important quality requirement (Van Buren *et al.*, 1986). Nevertheless, its acceptability for domestic cooking would be subject to local preferences. On the other hand, given their high hydration capacities, low incidence of splitting and relatively short cooking times, both the red-coloured genotypes (R_RED1 and R_SONG) would be suitable for industrial canning. The B_IPBB and N_ANAM genotypes, which exhibited poor soaking characteristics and required extended cooking times, are not desirable for boiling. Instead, they may find application in other food products such as extruded snacks, fermented food products or compound extraction (e.g., protein isolate or starch), but further research is required to assess the feasibility.

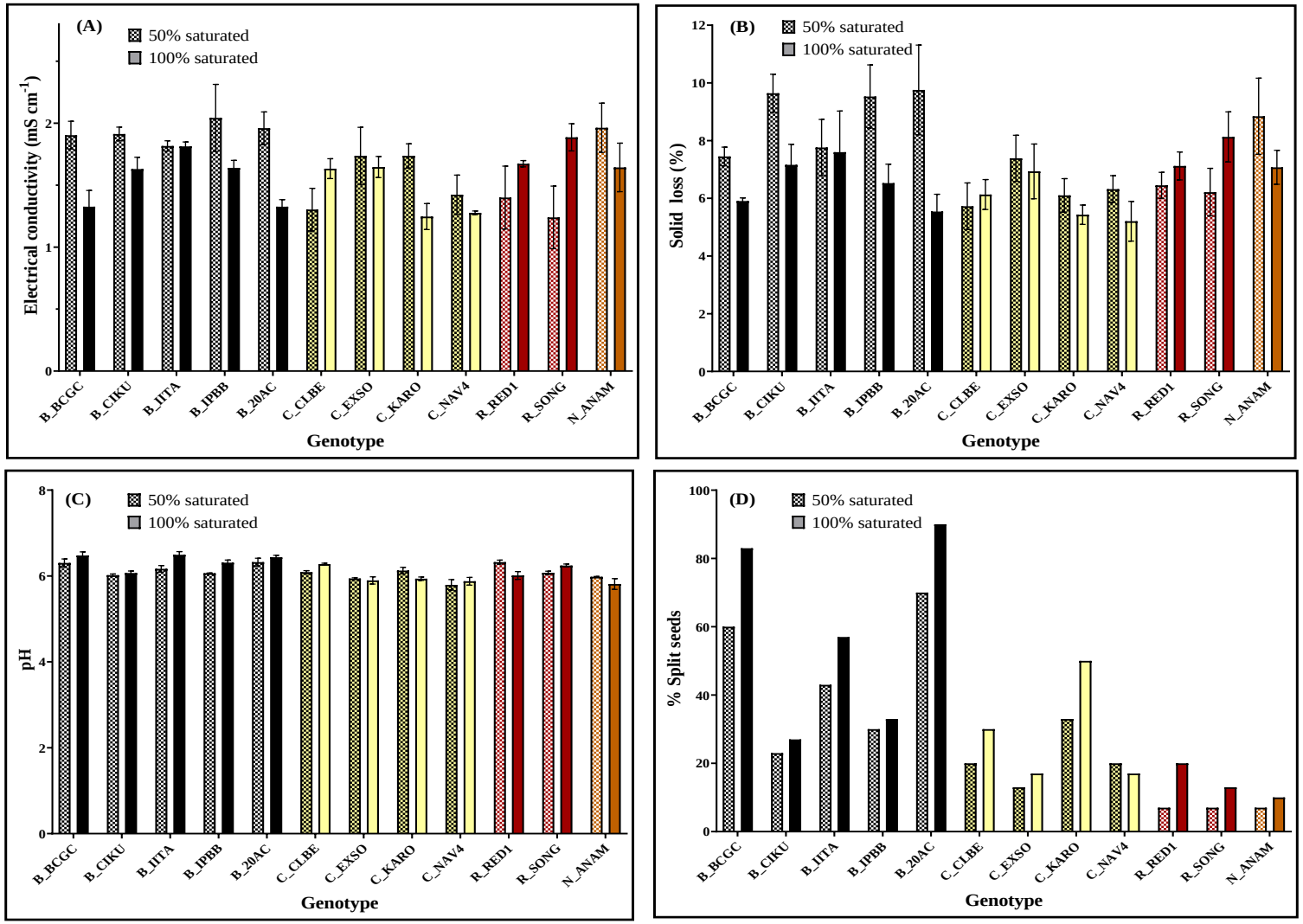

Figure 19 The EC (A), SL (B), and pH (C) of cooking liquid and percentage of split seeds (D) during cooking of Bambara groundnut genotypes after the seeds attained 50% (cross-pattern) and 100% (solid colour) hydration level. Vertical bars indicate standard deviation.

4.3.5 Correlations between physical and processing characteristics

The correlations between some physical, soaking, and cooking characteristics of Bambara groundnut are presented in Table 6. Of the three hydration parameters estimated by the kinetic model, the hydration rate constant (k) was negatively correlated with lag phase time (τ) ($r=-0.853$, $p<0.01$). This was expected since genotypes with rapid water uptake would have lower resistance to water absorption and would, therefore, experience a shorter duration of lag phase. The last parameter, equilibrium moisture content (M_{eq}), was an independent parameter and was not correlated ($p>0.05$) with the other two.

Percentage of seed coat was the only physical trait that was significantly correlated with the M_{eq} ($r=0.623$, $p<0.05$). This correlation could be explained by the composition of seed coat (Miano *et al.*, 2018), which is comprised mainly of non-starch polysaccharides (NSPs). High seed coat weights may indicate abundance of NSPs. These NSPs, which have high water holding capacity, may therefore allow the grain to absorb and retain more water.

It has been reported that seed size was inversely proportional to the hydration rate (Hsu, Kim and Wilson, 1983; Williams, Nakoul and Singh, 1983). Hsu *et al.* (1983) reasoned that small seeds tended to possess a higher surface-to-mass ratio, which could promote water migration through seed coat. However, this theory only holds true if the seed coats were permeable to water from the beginning of hydration, which was not the case for Bambara groundnut used in this study. Therefore, possibly due to the delayed permeability of the seed coat of Bambara groundnut, no significant correlations existed between the seed size and hydration kinetics.

There were, however, correlations found between seed shape and hydration parameters. Although seed shape might not dictate initial water entrance into the grain, it might affect water transport and distribution within the grain. For instance, the core of a flatter seed is expected to hydrate faster due to a shorter distance between the periphery and the centre of the seed. Contrary

to expectation, the hydration rate was positively correlated with sphericity ($p<0.01$), L/W ratio ($p<0.01$) and L/T ratio ($p<0.05$), indicating that a rounder seed tended to hydrate faster than a slender-shaped seed. This correlation implies that there may be other interfering factors, such as microstructural or compositional differences, that may outweigh the effect of seed shape. Wood *et al.* (2012) also found that rounded chickpea had higher hydration rate than the angular seeds, but the authors attributed the observation to differences in seed coat permeability.

In regard to seed coat colour, L* and b* values were positively correlated with k ($p<0.01$) but negatively correlated with τ ($p<0.01$). This is in line with the earlier observation that cream-coloured genotypes, which exhibited high L* and b* values, imbibed water at a faster rate and experienced shorter lag time.

Some anatomical characteristics, including seed coat thickness, palisade thickness, percentage of seed coat and relative size of hilum were also found to be associated to the soaking characteristics of Bambara groundnut. Hydration rate was inversely correlated with seed coat percentage ($r=-0.738$, $p<0.01$), seed coat thickness ($r=-0.784$, $p<0.01$), and palisade thickness ($r=-0.619$, $p<0.05$); whereas the lag phase time was positively correlated with seed coat percentage ($r=0.672$, $p<0.05$), seed coat thickness ($r=0.826$, $p<0.01$), and palisade thickness ($r=0.618$, $p<0.05$). This confirms previous findings indicating the key role of seed coat acting as a mechanical barrier to retard early water imbibition (Sefa-Dedeh and Stanley, 1979; Deshpande and Cheryan, 1986a). The correlation analysis also indicated that specific surface area of hilum was negatively correlated with the lag phase time ($r=-0.614$, $p<0.05$). This finding is in accordance with earlier discussion (Section 4.3.2.1) that hilum is the primary site of water absorption during the early stages of soaking and is of great importance in governing the initial water uptake. Interestingly, specific hilum surface area was negatively correlated with seed coat and palisade thickness ($r=-0.700$ and -0.673, respectively; $p<0.05$). It is possible that thick testae

and palisade layers, as well as small specific hilum area, act in synergy and contribute to impede initial hydration.

Since boiling involves heat transfer through conduction, the small-seeded genotypes were expected to be heated and cooked at a faster rate compared to the bigger seeds. Several studies reported seed size to be positively correlated with CT of other pulses (Williams, Nakoul and Singh, 1983; Giami and Okwechime, 1993; Black *et al.*, 1998). In the present study, a significant ($p<0.05$) positive correlation was observed between CT-50S and mean geometric diameter ($r=0.648$). However, once the seeds were soaked to saturation before cooking, the significant correlation between seed size and CT disappeared. This observation could be partially attributed to the high incidence of seed coat splitting in some large-seeded genotypes, namely B_BCGC and B_20AC (Figure 19). The splitting of testa partially removed the mechanical constraint to water uptake and grain swelling while exposing the cotyledons to cooking water, therefore shortening the CT. Furthermore, it is worth noting that the relationship between seed size and CT remains debatable, as studies also noted lack of correlation between the two traits (Black *et al.*, 1998; Yeung *et al.*, 2009; Wang *et al.*, 2010). In fact, the small-seeded cultivars of common bean (Paredes-López *et al.*, 1986) and pea (Wang, Daun and Malcolmson, 2003) was found to cook slower than the large-seeded cultivars. Therefore, seed size may not be a useful predictor for the cookability of legume seeds.

In contrast to popular beliefs that seeds with light-coloured testae tend to cook faster than their dark-coloured counterparts (Berchie *et al.*, 2010), no correlation was found between CIELAB L* and CTs of Bambara groundnut in the present study. Additionally, despite previous reports that suggest a relationship between thickness or weight percentage of seed coat and cookability of pulses (Muller, 1967; Bassett, Hooper and Cichy, 2021), this was not observed in the present study. These results suggest that seed coat colour and thickness are not important parameters affecting the cooking rate of Bambara groundnut.

Hydration rate during soaking was negatively and significantly correlated with CT-50S ($r=-0.579$, $p<0.05$), but was not associated ($p>0.05$) with CT-100S. A possible explanation is that genotypes which imbibe water at a faster rate during soaking tend to have a higher water absorption rate during cooking. If the seeds were not fully hydrated before cooking, hydration process would occur simultaneously with cooking. Thus, rapid influx of water during cooking would be crucial in facilitating the dissolution of middle lamella and the subsequent cell separation, as well as promoting starch gelatinisation and protein denaturation to render the seed cooked. However, when the seeds were soaked to saturation before cooking, the cellular components would have been fully hydrated, and thus, the rate of water uptake no longer exerted important influence on the CT. This finding corroborates the report by Jackson and Varriano-Martson (1981), who concluded that the HTC phenomenon was not associated with the behaviour of seed imbibition. The lack of correlation between soaking parameters and CT-100S indicates that other factors, such as chemical composition of the seed, may play a greater role in influencing the CT of fully hydrated seeds.

Table 6 Correlations between selected physical, soaking and cooking characteristics of Bambara groundnut (n=12).

	Meq	k	T	Dg	HSW	Sph	L/W	L/T	L*	b*	SCP	SCT	PalT	SSA_H	CT50S	CT100S
Meq	x	-	-	-	-	-	-	-	-	-	0.623*	-	-	-	-	-
k		x	-0.853**	-	-	0.808**	-0.756**	-0.676*	0.796**	0.694*	-0.738**	-0.784**	-0.619*	-	-0.579*	-
T			x	-	-	-0.813**	0.833**	0.620*	-0.875**	-0.833**	0.672*	0.826**	0.618*	-0.614*	-	-
Dg				x	0.934**	-0.589*	-	-	-	-	-	-	-	-	0.648*	-
HSW					x	-	-	-	-	-	-	-	-	-	-	-
Sph						x	-0.920**	-0.895**	0.866**	0.851**	-0.785**	-	-	-	-	-
L/W							x	0.654*	-0.932**	-0.932**	0.692*	-	-	-	-	-
L/T								x	-0.607*	-0.585*	0.721**	-	-	-	-	-
L*									x	0.976**	-0.777**	-0.809**	-0.641*	0.621*	-	-
b*										x	-0.671*	-0.710*	-	0.616*	-	-
SCP											x	0.673*	-	-	-	-
SCT												x	0.914**	-0.700*	-	-
PalT													x	-0.673*	-	-
SSA_H														x	-	-
CT50S															x	0.810**
CT100S																x

* and ** indicate significant at $p<0.05$ and $p<0.01$, respectively; - indicates not significant at $p<0.05$

Meq: equilibrium moisture content; k: hydration rate; T: time required to achieve half saturation; Dg: geometric mean diameter; HSW: 100-seed weight; Sph: sphericity; L/W: L/W ratio; L/T: L/T ratio; L* and b*: CIELAB scales; SCP: seed coat percentage; SCT: seed coat thickness; PalT: palisade thickness; SSA_H: specific hilum surface area; CT50S: cooking time of seeds pre-soaked to 50% saturation; CT00S: cooking time of fully hydrated seeds

4.4 Conclusion

Bambara groundnut genotypes showed considerable variations in physical, soaking and cooking characteristics. Nonetheless, the geometry and gravimetric traits of Bambara groundnut used in this study were comparable to those reported in the literature. Water uptake by the 12 Bambara groundnut genotypes was successfully modelled using a sigmoidal equation. Some physical characteristics and the anatomical features of seed coat and hilum may play a role in determining the hydration behaviour of Bambara groundnut seeds. Seed coat colour was indicative of hydration behaviour but was not associated with cooking time. On the other hand, seed size, seed shape and hydration rate were among the important attributes influencing cooking time of partially hydrated seeds. The cooking time of fully hydrated seeds, however, did not appear to be significantly correlated with any of the physical traits and hydration kinetics parameters. Increased solute leaching during soaking, which indicates seed deterioration, was an important indicator of HTC phenomenon, confirming the cell membrane damage hypothesis.

The lack of interrelationships between cooking time of fully hydrated Bambara groundnut seeds and (1) physical, (2) soaking, and (3) microstructural properties necessitate a further study into the chemical factors associated with the HTC phenomenon. Among the genotypes studied, the fully hydrated seeds of C_KARO and R_SONG had substantially shorter cooking times (38-43 min) compared to B_IPBB and N_ANAM (80-120 min). Thus, these four genotypes were selected for the subsequent study to examine the key chemical components that could explain the genotypic differences in cooking quality.

Chapter 5: Comparison of protein, starch, and cell wall components between easy-to-cook and hard-to-cook Bambara groundnut genotypes

5.1 Introduction

Bambara groundnut is commonly boiled before consumption to render the seeds edible. The hydrothermal process leads to solubilisation of middle lamella and separation of adjacent cotyledon cells, accompanied by gelatinisation of starch granules and denaturation of proteins within individual cells. Previous studies have suggested that the rates of middle lamella dissolution (Bernal-Lugo *et al.*, 1997; Chigwedere *et al.*, 2018), starch gelatinisation, and protein denaturation (Garcia-Vela and Stanley, 1989a; Garcia-Vela, del Valle and Stanley, 1991; del Valle *et al.*, 1992) may be associated with the cooking time of pulses.

The middle lamella, an intercellular layer that cements plant cells together, is composed mainly of pectin (Reyes-Moreno, Paredes-López and Gonzalez, 1993). Texture softening of legume seeds during cooking is primarily attributed to the solubilisation of pectin in the middle lamella, thereby weakening the cell-to-cell adhesion and causing cell separation (Chigwedere *et al.*, 2019). The pectin-cation-phytate hypothesis postulates that during seed ageing, de-methyl-esterification of pectin produces free carboxyl groups on the pectin whilst dephosphorylation of phytate gives rise to free divalent cations (e.g., Ca^{2+} and Mg^{2+}). The two intermediate products may then crosslink in the middle lamella to form water-insoluble pectate complexes. These pectate salts strengthen the cell walls and the intercellular adhesion in the middle lamella, thereby restricting cell separation during cooking and consequently resulting in firmer seed texture.

Additionally, starch and protein have been suggested as potential factors influencing the cookability of pulses. During cooking process, starch granules undergo structural disorganisation

and transformation from an ordered semi-crystalline structure to a disordered amorphous state, while proteins undergo transition from a native to a denatured conformation. These changes are essential to improve the digestibility of these macromolecules (Oyeyinka, Pillay and Siwela, 2019). Thermal stability of the starch and protein has been implicated with the HTC phenomenon of pulses (Liu, McWatters and Phillips, 1992; Reyes-Moreno *et al.*, 1994). Liu *et al.* (1992) suggested the involvement of protein in HTC phenomenon and proposed the protein insolubilisation hypothesis. As demonstrated in Chapter 4, the starch granules in the cotyledon cells of Bambara groundnut are surrounded by a protein matrix. According to the hypothesis, protein insolubilisation may occur during the ageing process, causing the protein to coagulate and denature at a lower temperature during cooking. During cooking, the denatured protein may then form a hydrophobic barrier to retard water uptake by starch, or a physical barrier to restrict starch swelling., thereby impeding starch gelatinisation (Liu, McWatters and Phillips, 1992).

Although several hypotheses have been proposed to explain the HTC phenomenon, a consensus on the cause of HTC phenomenon has not been reached. Identifying the chemical components that play a significant role in the development of HTC phenomenon is imperative in furthering the understanding of the underlying mechanisms, which in turn is crucial to establish appropriate processing methods to shorten the cooking time. Chapter 4 demonstrated a wide variation in cooking times between Bambara groundnut genotypes. In order to further explore the mechanisms leading to hard-to-cook phenomenon, this study was undertaken to determine the variations in chemical characteristics between the easy- and hard-to-cook Bambara groundnut genotypes.

5.2 Materials and methods

5.2.1 Materials

Following the screening trial in Chapter 4, four genotypes (Section 3.1) were selected for this study: C_KARO and R_SONG as the easy-to-cook (ETC) genotypes, whilst B_IPBB and N_ANAM as the hard-to-cook (HTC) genotypes. Seed samples were ground using an ultra-centrifugal mill (ZM 200, Retsch, Germany). Ground powder was passed through 0.21 mm (No. 70) mesh sieve and packed in zip-lock bags. Samples were kept at room temperature with silica gel until analysis.

5.2.2 Protein

5.2.2.1 Crude Protein

The crude protein content of the flour was determined by Kjeldahl method (Buchi, Switzerland). Briefly, a 0.2 g of sample, two titanium tablets and 15 mL concentrated sulfuric acid were placed in each digestion tube. Digestion was performed at 380°C for 90 min until the tube was clear with no charred material remaining. Distillation was carried out with 100 mL of distilled water, 90 mL of 32% (w/v) NaOH and 50 mL of 4% (w/v) boric acid. The final solution was titrated with 0.1 M HCl to pH 4.65 and the volume of HCl added was recorded.

$$\%\ Protein = \frac{V\ (Sample - Blank) \times n \times M_N}{m_{sample}} \times PF \qquad (15)$$

where V = titrant volume (ml); n = normality of HCl (mol/ml); M_N = molecular weight of nitrogen (14 g/mol); m_{sample} = sample weight (g); PF = protein factor (6.25)

5.2.2.2 Protein Solubility

Protein solubility was determined according to the method of Liu *et al.* (1992). One gram of flour was mixed with 20 mL of deionised water. The suspension was stirred on a magnetic hotplate stirrer for 45 min at room temperature, then centrifuged at 9800 ×g for 15 min. Protein content in

the supernatant, analysed as water-soluble protein, was determined by the Kjeldahl method. Protein solubility was expressed as the percentage of the protein in supernatant to the crude protein in flour.

5.2.3 Thermal properties of flour

Thermal properties of Bambara groundnut flour were studied using DSC (Section 3.2.6).

5.2.4 Pectin composition

5.2.4.1 Extraction of alcohol-insoluble residue (AIR)

Cell wall material was isolated as AIR according to Njoroge *et al.* (2014) and Yi *et al.* (2016) with minor modifications. The samples were first gelatinised to allow enzymatic reaction. Two grams of flour was mixed with 50 mL of 0.1 M sodium phosphate buffer (pH 6.0) and incubated in a water bath at 96°C for 10 mins with constant stirring. The high-temperature, short-time approach was employed to ensure complete starch gelatinisation and protein denaturation while minimising changes in pectin structure and solubility. This was followed by a series of enzymatic treatment to remove starch and proteins. The suspension was cooled to 65°C by adding 50 mL of 0.1 M sodium phosphate buffer (pH 6.0). Subsequently, 1 mL of α-amylase enzyme (410 U/mL buffer) Type II-A from *Bacillus species* (Sigma-Aldrich Inc., USA) was added, and the mixture was incubated at 65°C for 1 h in a water bath. The pH was adjusted to 7.5 with 1 M NaOH and then, 0.4 mL of protease Type VIII enzyme (61.5 U/mL buffer) from *Bacillus licheniformis* (Sigma-Aldrich Inc., USA) was added and the suspension was incubated at 60°C for 1 h. Finally, after pH adjustment to 4.3 (using 1 M HCl), the suspension was incubated with 1.5 mL of amyloglucosidase enzyme (≥260 U/mL buffer) from *Aspergillus niger* (Sigma-Aldrich Inc., USA) at 60°C for 1 h. Throughout the enzymatic digestion process, beakers were covered with aluminium foil and were manually swirled every 15 min.

After cooling, the suspension was centrifuged at 10,000 ×*g* for 20 min. To remove soluble sugars, the pellet was first mixed with 64 mL of 80% alcohol. The suspension was vortex mixed and then filtered (Whatman No 1 filter paper). The extraction was repeated with 32 mL of 80% alcohol. Finally, the residue was mixed with 32 mL of acetone at 4°C for 10 min. After filtration, the residue was dried at 40°C for 16 h to yield AIR. The dry AIR was ground using a mortar and pestle, weighed, and stored at -18°C until analysis. Extraction was performed in duplicate.

5.2.4.2 Fractionation of pectin

The AIR was fractionated into water-soluble pectin (WSP) and chelator-soluble pectin (CSP) as described by Njoroge *et al.* (2014). To extract WSP, the AIR (0.1 g) was mixed with 20 mL boiling distilled water and boiled for 5 min over a hotplate. The mixture was cooled in water bath and filtered using Whatman No. 1 paper. The filtrate was collected, pH adjusted to 6.5 with 0.01 M NaOH, volume made up to 20 mL with distilled water, and was designated as WSP. The residue was then incubated with 20 mL of 0.05 M cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA) in 0.1 M potassium acetate buffer (pH 6.5) for 6 h at 28°C. The mixture was filtered, the filtrate volume was adjusted to 20 mL with CDTA solution and then labelled as CSP. Fractionation was performed in triplicate. For each fraction obtained, three pectin content determinations were performed.

5.2.4.3 Determination of pectin content

The pectin content was estimated as galacturonic acid (GalA) content according to Blumenkrantz and Asboe-Hansen (1973). Samples (0.2 mL) and 1.2 mL of sulfuric acid (98%) containing 0.0125 M sodium tetraborate were added to test tubes, vortex mixed and incubated in water bath at 98°C for 5 min. After cooling in water-ice bath for 10 min, 20 µL of 0.5% NaOH containing 0.15% 3-phenylphenol (Sigma-Aldrich Inc., USA) was added. The mixture was vortex mixed and the absorbance was read at 520 nm within 5 min using UV-Vis spectrophotometer (Libra S12, Biochrom Ltd., UK). For blank, 3-phenylphenol solution was replaced by 0.5% NaOH. A

standard curve was constructed using mono-GalA (Sigma-Aldrich Inc., USA) (1-60 µg/mL; R^2 = 0.9826) and results were expressed as mg GalA g^{-1} AIR.

To determine the total GalA content of AIR, the samples were first hydrolysed in concentrated sulfuric acid as described by Ahmed and Labavitch (1977). The GalA concentration of the hydrolysate was then determined as described above.

5.2.4.4 Degree of Methyl-esterification (DoM) of pectin

The DoM of pectin was determined based on the FTIR spectra collected, according to Kyomugasho *et al.* (2015). The ratio of peak intensity at 1745 cm^{-1} to the sum of peak intensities at 1745 and 1630cm^{-1} of AIR samples was calculated, then converted to DoM (%) using the calibration curve. Spectra were not deconvoluted.

5.2.5 Structural organisation of protein, starch, and AIR

FTIR study was performed on the flour and alcohol insoluble residue of Bambara groundnut. FTIR spectra were obtained using a FTIR spectrophotometer (Frontier, PerkinElmer, USA) equipped with attenuated total-reflectance (ATR) accessory (GladiATR, PIKE Technologies, USA). Powdered samples were placed on the diamond crystal plate (sampling window) and consistent pressure was applied using a pressure clamp. The spectra were recorded in the range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1}, against a background spectrum measured on an empty sample plate. Samples were analysed in triplicate, and 100 scans were recorded for each measurement to reduce the noise-to-signal ratio (Kyomugasho *et al.*, 2015). Peak analysis was performed using OriginPro2021b (Learning Edition) (OriginLab Corporation, USA).

5.2.5.1 Protein secondary structure

FTIR spectra of flour in the 1700-1600 cm^{-1} region were subjected to baseline correction, followed by Fourier Self Deconvolution (gamma = 10; smoothing factor = 0.25). Curve fitting was performed to identify the peaks and to determine the corresponding peak area. Gaussian line

shape was assumed (Carbonaro *et al.*, 2008). The relative composition of each secondary structure was calculated as the ratio of band area of each component to the total band area of all components.

5.2.5.2 Degree of order of starch

FTIR spectra of flour in the 1200-800 cm^{-1} region were subjected to baseline correction, followed by Fourier Self Deconvolution (gamma = 15; smoothing factor = 0.25). The height ratio of peak at 1047 and 1016 cm^{-1} for each spectrum was calculated (Maaran *et al.*, 2014).

5.2.6 Statistical analysis

Data for chemical composition and thermal analysis were subjected to one-way ANOVA using SPSS Version 28 (IBM Corporation, USA) to determine whether there is a statistical difference between genotypes. When significant differences ($p<0.05$) were found, means were compared and separated by Tukey *post hoc* test.

5.3 Results and discussion

5.3.1 Protein solubility

The average crude protein contents of the four Bambara groundnut genotypes ranged from 21.93 (N_ANAM) to 23.53% (dwb) (C_KARO) (Table 7), comparable to those published in the literature (Nti, 2009; Oyeyinka *et al.*, 2017; Halimi *et al.*, 2019). However, contrary to previous studies who reported significant varietal differences in the protein content of Bambara groundnut (Nti, 2009; Adeleke, Adiamo and Fawale, 2018), there were no significant differences ($p>0.05$) between the genotypes in this study.

Changes in protein solubility have been associated with the development of HTC phenomenon of legume seeds. Several studies have demonstrated a decrease in protein solubility with a concomitant increase in the HTC of pulses during improper storage (Hentges, Weaver and Nielsen, 1991; Liu, McWatters and Phillips, 1992; Koriyama *et al.*, 2017). Saio *et al.* (1980) reported that extended storage under high temperature (35°C) and relative humidity (RH) (80%) was the major contributing factor to decreased protein solubility in soybean. This phenomenon may be explained by the biochemical changes that occur during the ageing process, such as hydrolysis of lipids into fatty acids, or protein into amino acids, which are favoured under high temperature and RH storage conditions (Liu, McWatters and Phillips, 1992). These reactions cause acidification of bean tissue and push the tissue pH closer to the isoelectric pH of legume proteins, thus lowering the protein solubility. The increase in insoluble protein content under adverse storage conditions could also be attributed to the formation of lignified proteins which are insoluble in water (Hohlberg and Stanley, 1987).

In this study, the protein solubility, measured as water-extractable protein, varied between 33.74 (R_SONG) and 38.09% (C_KARO). Similar values have been reported for Bambara groundnut flour (Adebowale, Schwarzenbolz and Henle, 2011), but the authors did not describe

the storage period and conditions of their samples. Although the samples used in this study had been stored for approximately 36 months, the protein solubility values were considerably higher than that found in cowpea by Liu *et al.* (1992), who documented a protein extractability of 11.2% after 18 months of adverse storage (30°C/64% RH). This may be attributed to the differences in species and storage conditions. Additionally, there was no significant ($p>0.05$) genotypic variation for protein solubility despite the wide variation in cooking time. In summary, there is no clear relationship between protein solubility and HTC phenomenon of Bambara groundnut in this study, suggesting that protein solubility might not be responsible for the prolonged cooking time of HTC genotypes.

Table 7 Crude protein content and solubility of different Bambara groundnut genotypes.

Genotype	Crude protein (% dwb)	Protein solubility (%)
C_KARO	23.53 ± 2.27^{a}	38.09 ± 0.57^{a}
R_SONG	22.28 ± 0.06^{a}	33.74 ± 1.53^{a}
B_IPBB	22.16 ± 2.42^{a}	33.78 ± 7.61^{a}
N_ANAM	21.93 ± 2.60^{a}	38.00 ± 0.62^{a}

Readings are means ± SD (n=3). Values with different superscript letters within a column are significantly different ($p<0.05$) with Tukey *post hoc* test.

5.3.2 Thermal analysis

Thermal analysis was performed to monitor the thermal transitions of flour as a function of temperature, and to examine the association between thermal stability and cooking time of Bambara groundnut. The differential scanning calorimetry (DSC) thermograms of Bambara groundnut flours exhibited a single endothermic peak at around 80°C (Figure 20), consistent with the thermal curves observed in cowpea (Henshaw *et al.*, 2003) and other varieties of Bambara groundnut (Gwala *et al.*, 2019) flours. In contrast, the thermograms of other pulse flours (e.g. *Lens* spp., *Vigna* spp., *Vicia* spp., and *Phaseolus* spp.) were reported to show two distinct endothermic transitions, in which the lower and the higher transition peaks were ascribed to starch gelatinisation and protein denaturation, respectively (Sosulski *et al.*, 1985). Some studies attributed the single endotherm exhibited by Bambara groundnut solely to starch gelatinisation (Mubaiwa *et al*., 2018; Gwala *et al.*, 2019). However, considering that the seed contains both starch and protein at appreciable levels (Halimi *et al.*, 2019), a more plausible explanation would be that the single major peak was the result of a composite thermal event consisting of starch gelatinisation and protein denaturation, as described by Henshaw *et al.* (2003).

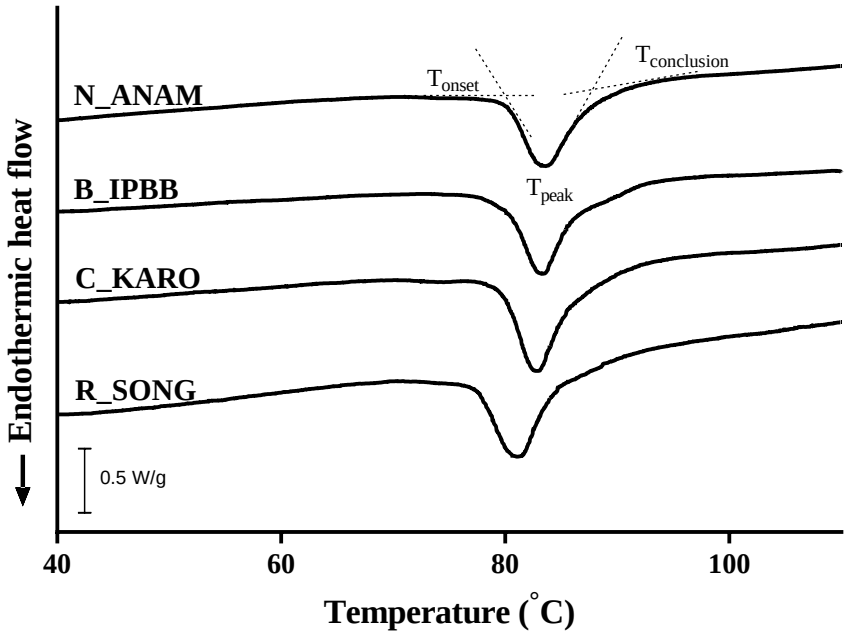

Figure 20 DSC thermograms of four Bambara groundnut genotypes.

The thermal properties of the four Bambara groundnut flours are summarised in Table 8. The temperatures at which thermal transition occurred were described as the onset (T_o), peak (T_p) and conclusion (T_c) temperatures, whereas the enthalpy change (ΔH) was a measurement of energy requirement for the thermal event. In the present study, the T_o (75.1-77.7°C) and ΔH (5.76-7.61 J g^{-1}) values obtained were comparable to those reported for Bambara groundnut by other authors (67.8-77.1°C and 4.62-9.37 J g^{-1}, respectively; Kaptso *et al*., 2015; Mubaiwa *et al*., 2018; Chinma *et al*., 2021), but the T_p (80.1-83.6°C) and T_c (87.7-92.9°C) values were higher than those recorded by these authors (72.7-81.3°C and 83.1-87.1°C, respectively). These differences may be due to varietal difference of Bambara groundnut used, but the possible effect of storage time should not be overlooked (Garcia-Vela and Stanley, 1989a).

Previous works have reported contradictory results of the effect of HTC phenomenon on the thermal transition temperatures of common beans (Hohlberg and Stanley, 1987; Garcia-Vela and Stanley, 1989a; Paredes-López, Maza-Calviño and González-Castañeda, 1989; Reyes-Moreno *et al.*, 1994; Koriyama *et al.*, 2017). In this study, there was no relationship between T_o and seed cookability among these genotypes. However, the T_p and T_c values of the HTC genotypes were significantly ($p<0.05$) higher than those of the ETC genotypes, indicating abundance of structurally stable biomolecules (starch and protein) which caused them to be more resistant to thermal transition. It has been suggested that Bambara groundnut starch with low amylose contents and abundant of long amylopectin chains may exhibit a greater thermal stability (Oyeyinka, Singh and Amonsou, 2017). The constituents of protein components and tertiary conformation of polypeptides may also influence the T_p value of protein fractions (Adebowale, Schwarzenbolz and Henle, 2011; Alabi *et al.*, 2020). The wide range of transition temperature (T_{onset}-$T_{conclusion}$) in HTC genotypes (Table 8) reflects the higher heterogeneity of intermolecular binding forces and structural organisation of the starch and protein among the HTC genotypes, thus causing greater variations in heat stability.

The HTC genotypes also exhibited significantly ($p<0.05$) greater ΔH, indicating that more energy is required to break the intermolecular hydrogen bonds of starch granules and protein structures to achieve starch gelatinisation and protein denaturation, which may also partly explain their longer cooking time. Sánchez-Arteaga *et al.* (2015) associated a higher protein denaturation temperature and enthalpy with the HTC varieties of common bean, and speculated that the heat-resistant proteins could be a dominant factor affecting bean cooking time. This postulation is not surprising since protein denaturation of common bean has been reported to occur at the later stages of cooking, whereas starch gelatinisation was completed during the early stages (Garcia-Vela and Stanley, 1989a). It is, however, not possible to assess the contribution of protein denaturation process in the present study since the endotherms of starch gelatinisation and protein denaturation appeared as a single peak.

Table 8 DSC thermal properties of four Bambara groundnut genotypes.

Genotype	Thermal transitions (°C)				Enthalpy, ΔH (J g^{-1})
	T_{onset}	T_{peak}	$T_{conclusion}$	Range ($T_c - T_o$)	
C_KARO	76.9 ± 0.3^{ac}	81.6 ± 0.2^{a}	87.8 ± 0.6^{a}	10.9 ± 0.4^{a}	6.22 ± 0.19^{a}
R_SONG	75.1 ± 1.0^{b}	80.1 ± 0.1^{b}	87.7 ± 1.1^{a}	12.6 ± 1.1^{a}	5.78 ± 0.28^{a}
B_IPBB	76.2 ± 0.3^{ab}	82.4 ± 0.3^{c}	91.4 ± 1.1^{b}	15.2 ± 0.9^{b}	7.61 ± 0.55^{b}
N_ANAM	77.7 ± 0.4^{c}	83.6 ± 0.5^{d}	92.9 ± 0.9^{b}	15.2 ± 1.3^{b}	7.44 ± 0.51^{b}

Values are means ± SD (n=3). Values with different superscript letters within a column are significantly different ($p<0.05$) with Tukey *post hoc* test.

5.3.3 Cell wall pectin composition

The yields of alcohol-insoluble residue (AIR) from ETC and HTC genotypes were statistically similar, ranging from 12.79-14.45% dwb (Table 9). These values were substantially lower than those reported for common bean (>30%) despite using the same extraction protocol (Njoroge *et al.*, 2014). This could be due to relatively lower amounts of seed coat present in the Bambara groundnut flours because the seed coat fraction, which was harder to be milled into finer particles, was prevented from passing through the 0.21 mm mesh size. Yi *et al.* (2016) reported that AIR extracted from the cotyledon tissue of common bean had a lower yield at approximately 20% dwb.

Table 9 Yield, GalA content, and degree of methyl-esterification of AIR extracted from different Bambara groundnut genotypes.

Genotype	AIR yield (% dwb)	GalA content (mg g^{-1} AIR)	Degree of methyl-esterification (DoM, %)
C_KARO	12.79 ± 1.33^{a}	60.34 ± 2.79^{ab}	44.62 ± 2.84^{ab}
R_SONG	13.02 ± 0.21^{a}	63.64 ± 3.41^{a}	56.44 ± 7.65^{c}
B_IPBB	14.45 ± 0.27^{a}	56.22 ± 2.23^{b}	37.01 ± 1.37^{a}
N_ANAM	14.21 ± 0.81^{a}	62.30 ± 2.46^{a}	48.17 ± 2.55^{bc}

Values are means ± SD (n=3). Values with different superscript letters within a column are significantly different ($p<0.05$) with Tukey *post hoc* test.

Since pectin is a polysaccharide composed mainly of galacturonic acid (GalA), its content can be estimated by determining the amount of GalA present. Table 9 shows the total GalA content in AIR of different Bambara groundnut genotypes. The concentration of GalA was similar for all genotypes, except that B_IPBB had significantly ($p<0.05$) lower content than R_SONG and N_ANAM. The GalA content did not show a trend with respect to ease of cooking,

suggesting that despite its vital role in providing mechanical strength to the cell wall, the total pectin content in the cell wall materials was not the primary determinant of the cooking quality of Bambara groundnut. This finding is in agreement with studies on other pulses (Shehata, El-Shimi and Mesallam, 1985; Bhatty, 1990; Wainaina *et al.*, 2022).

Pectin present in the AIRs was sequentially extracted into two fractions based on the pectin solubility. The AIR was first extracted in boiling water as water-soluble pectin (WSP). Heating the AIR in boiling water disrupted the hydrogen bonds and released the weakly-bound pectic substances from the cell wall. Following that, treatment of the remaining AIRs with CDTA solubilised the pectic substances that were held in the cell wall by strong ionic bonds. This is because CDTA, as a chelating agent, is capable of sequestering divalent cations (e.g., Ca^{2+} and Mg^{2+}) from the pectate complexes (e.g., Ca-pectate), thus liberating the pectic molecules. It has been suggested that the CDTA-soluble pectin (CSP) fractions are predominantly derived from the middle-lamella region (Ryden and Selvendran, 1990), and may therefore play an important role in the cookability of legume seeds.

The amounts of WSP and CSP in the AIR extracted from four Bambara groundnut genotypes are presented in Figure 21. The WSP fractions of ETC genotypes contained significantly ($p<0.01$) higher concentrations of GalA (approximately 30% higher) than those of the HTC genotypes, indicating a higher abundance of pectic substances that are loosely bound to the cell wall of the ETC genotypes. During hydrothermal treatment, this pectin fraction can easily dissolve in hot water, thus promoting cell separation through the middle lamella, leading to a higher rate of texture softening. Conversely, the HTC genotypes exhibited significantly ($p<0.05$) higher levels of GalA in the CSP fractions, about 20% higher than the ETC genotypes. This indicates higher contents of ionically cross-linked insoluble pectate salts in the cell walls of HTC genotypes, which strengthens intercellular adhesion and delays pectin solubilisation during cooking. These results are in accord with other studies that showed a higher concentration of CSP

was associated with greater hardness level of cooked beans (Bernal-Lugo *et al.*, 1997) and an increased water solubility of pectin shortened the bean cooking time (Ogundele and Emmambux, 2018). In the present study, the HTC genotypes also had significantly ($p<0.01$) higher amounts of GalA in the CSP fractions than in the WSP fractions, suggesting a higher proportion of insoluble and strongly bound pectin in the middle lamella of these genotypes.

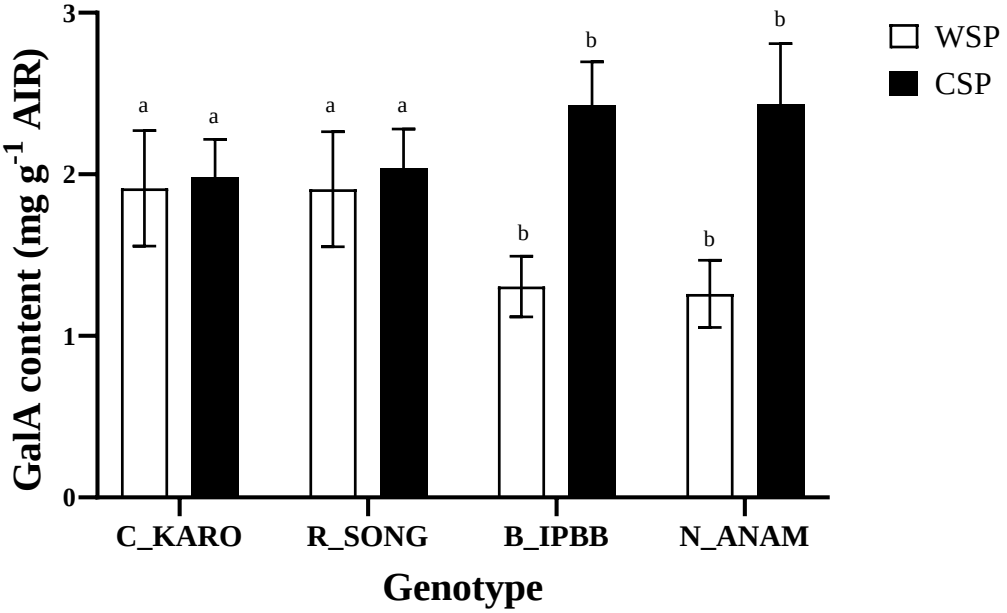

Figure 21 Galacturonic acid content in water-soluble pectin (WSP) and chelator-soluble pectin (CSP) fractions of Bambara groundnut genotypes. Values are means ± SD (n=9). Columns of the same colour with different letters are significantly different ($p<0.05$) with Tukey *post hoc* test.

In addition to the solubilisation process, pectin is subjected to depolymerisation during thermal processing, which also leads to texture softening during cooking (Liu and Bourne, 1995). This process, known as β-elimination, involves the breaking down of glycosidic bond of the methyl-ester group in pectin, resulting in degradation of pectic polymers into smaller molecular weight products. Thus, pectin with a higher degree of methyl-esterification (DoM) is more susceptible to β-elimination and therefore degrades more during cooking than pectin with a lower DoM. This, in turn, would accelerate the rate of bean softening during cooking. Another

mechanism that considers the involvement of DoM in HTC phenomenon is the pectin-cation-phytate model. The model postulates that the DoM of pectin decreases during ageing due to removal of methyl-ester residues from pectin by enzyme pectin methylesterase (PME) (Jones and Boulter, 1983). This de-methyl-esterification process is crucial in creating more binding sites (free carboxyl groups) for divalent cations cross-linking, thereby inducing hardening of bean. Both β-elimination and pectin-cation-phytate hypotheses point to a lower pectin DoM in HTC pulses.

In the present study, the DoMs of Bambara groundnut AIR, ranging from 37-56% (Table 9), are comparable to those of common bean (Njoroge *et al.*, 2014; Yi *et al.*, 2016). Contrary to the β-elimination hypothesis, there was no observed association between bean cookability and DoM of pectin, implying that the rate of β-elimination is unlikely to account for the differences in bean softening rate during cooking. Similarly, Chigwedere *et al.* (2019) found no evidence of pectin depolymerisation during boiling of common bean. From the perspective of pectin-cation-phytate hypothesis, the DoM of pectin was expected to be inversely related to the level of chelator-soluble pectic substances. This, however, was not the case in the current study, implying that the DoM of pectin might not be an indicator of pectin insolubilisation taking place during storage. Studies on cowpea (Liu, Phillips and Hung, 1992), lentil (Bhatty, 1990) and common bean (Chigwedere *et al.*, 2019) also reported lack of relationship between HTC bean and DoM of pectin. Likewise, other studies failed to establish the role of enzyme PME in hardening phenomenon of common bean (Mafuleka *et al.*, 1991) and cowpea (Liu, Phillips and Hung, 1992). Although it was not possible to evaluate the influence of storage time and conditions on the changes in the pectin DoM in this study, previous works on common bean found no major changes in pectin DoM following ageing process (Chen *et al.*, 2021; Wainaina *et al.*, 2022). These results suggest inadequacy of pectin-cation-phytate model to explain the development of HTC phenomenon in Bambara groundnut.

5.3.4 Fourier transform infrared spectroscopy (FTIR)

A recent study highlighted the possibility of utilising near-infrared spectroscopy in predicting the susceptibility of common bean to develop HTC (Wafula *et al.*, 2021). However, there are limited studies reporting the application of mid-infrared spectroscopy on the HTC phenomenon. Hence, the FTIR study was undertaken to explore the differences in molecular structure conformation between ETC and HTC genotypes.

5.3.4.1 Bambara groundnut flour

Figure 22 shows the spectroscopic profiles for the Bambara groundnut flours in the region between 3000 to 800 cm^{-1}. Although the genotypes exhibited different physical, hydration and cooking characteristics, the spectral patterns of the flours appeared quite similar.

The two narrow and relatively intense bands that occurred just below 3000 cm^{-1}, centred at about 2924 and 2853 cm^{-1}, might suggest the presence of long-chain linear aliphatic compounds (Coates, 2006), probably arising from lipid compounds. The asymmetric C-H stretch of methylene group (2924 cm^{-1}) was more intense than the symmetric vibration of methylene group (2653 cm^{-1}) for all samples. The absorption peak located at around 1745 cm^{-1} may be attributed to C=O stretching of triglycerides.

The amide I band (1700-1600 cm^{-1}) corresponds to the C=O stretching vibration of amide group, whereas the amide II band (1575-1480 cm^{-1}) originates from the C-N stretching and N-H bending (Kong and Yu, 2007). Amide I band has implications for the secondary structure of protein, which will be discussed in the following section (Section 5.3.4.2). Amide II band is more complex and conformationally less sensitive, and thus is not widely used for protein conformation studies (Kong and Yu, 2007).

The bands between 1200-900 cm^{-1} represent the total carbohydrate region. The prominent peak centred at 994 cm^{-1} was likely derived from the C-O stretch of starch (Kaptso *et al.*, 2015).

An intense band in the region can also be observed in the FTIR spectra of Bambara groundnut starch (Kaptso *et al.*, 2015; Maphosa, Jideani and Ikhu-Omoregbe, 2022). The bands at 1048 and 1016 cm^{-1} are associated with the crystalline and amorphous regions of starch, respectively, and thus, the ratio of absorbances at 1047/1016 cm^{-1} can be used as an indicator of the ordered crystalline structure in starch (Maaran *et al.*, 2014). The ratio of 1047/1016 cm^{-1} of C_KARO, R_SONG, B_IPBB and N_ANAM was 0.903, 0.931, 0.937, and 0.913, respectively, indicating a higher level of ordered structure in B_IPBB starch fraction, followed by R_SONG, N_ANAM and C_KARO. These values were lower than those found in Bambara groundnut starch (0.97-1.04; Kaptso *et al.*, 2015), but higher than those reported for other pulse starches (0.813-0.901; Maaran *et al.*, 2014). The molecular order of starch did not show a trend to distinguish the difference between the ETC and HTC genotypes. Nevertheless, it is worth noting that this ratio could only represent the short-range molecular structure (local order of double helix) on the surface of starch granules, since the mid-infrared wavelength could not penetrate through the granules (Maaran *et al.*, 2014).

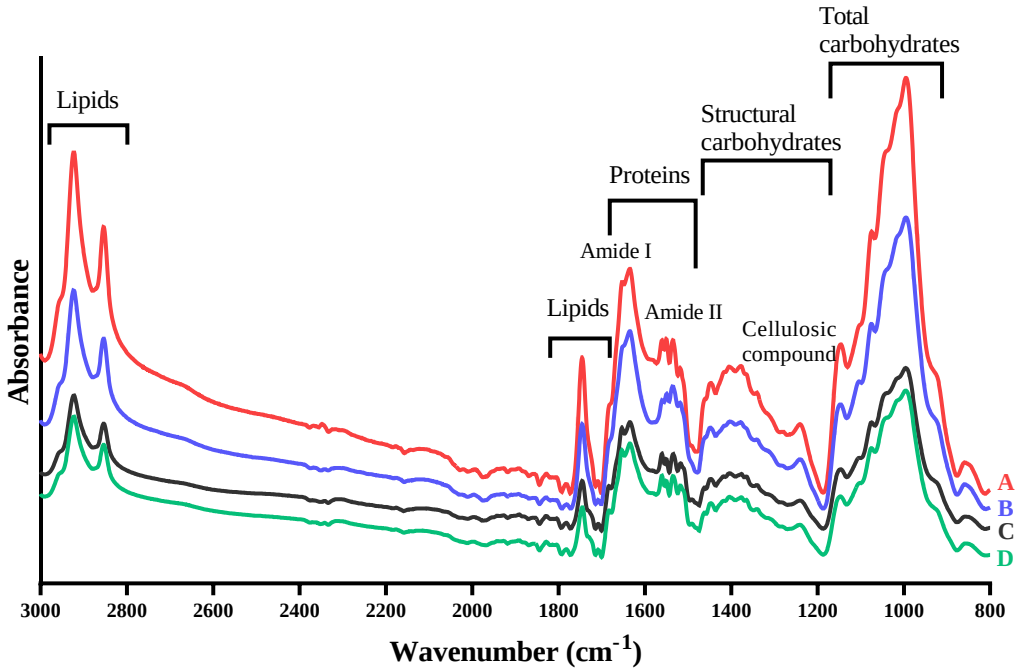

Figure 22 ATR-FTIR spectra of Bambara groundnut flours in the 3000-800 cm^{-1} region. (A) R_SONG; (B) C_KARO; (C) B_IPBB; (D) N_ANAM.

5.3.4.2 Protein secondary structure

The amide I region (1700-1600 cm^{-1}) of FTIR spectra was used in the determination of protein secondary structure. An example of the deconvoluted infrared spectrum after curve-fitting is presented in Appendix 3. Five peaks were found in the deconvoluted spectra and were assigned according to Table 10.

Table 10 Deconvoluted amide I frequencies assigned to protein secondary structure.

Frequency (cm^{-1})		Assignment
Literature[a]	Experiment[b]	
1615-1618	1612-1614	Inter-molecular aggregate (A1)
1630-1638	1634-1635	β-sheet
1650-1660	1654-1655	α-helix
1660-1672	1667	Turns
1682-1692	1683-1684	Intra-molecular aggregate (A2)

[a] Data obtained from Carbonaro *et al.* (2008), Long *et al.* (2015) and Parmar *et al.* (2017)

[b] Data obtained from this study

Quantification of the relative contents of the individual conformations revealed that the secondary structure of Bambara groundnut protein was dominated by β-sheet (37-40%), followed by α-helix (21-22%), A2 (18-19%), turns (9-11%) and A1 (9%) (Figure 23). Earlier studies on protein concentrates (Mune Mune and Sogi, 2016) and protein isolates (Ngui *et al.*, 2021) extracted from Bambara groundnut also reported that their conformations were primarily β-sheet structure. As with other legume seeds, vicilin is the major storage protein in Bambara groundnut (Diedericks *et al.*, 2019). The vicilin fraction extracted from Bambara groundnut was also found to contain high proportions of β-sheet and α-helix when analysed by circular dichroism (Arise *et al.*, 2017). Moderate contents of A2 in all genotypes may suggest considerable amounts of partially folded proteins and could be an indication of denatured protein (Kong and Yu, 2007).

The protein spectrum of common bean and lentil also showed moderate contribution (23-26%) by A2 component (Carbonaro *et al.*, 2008).

The secondary conformation of protein is associated with its functional properties. For instance, it has been observed that the solubility of Bambara groundnut protein decreased with increasing contents of β-sheet (Mune Mune, Sogi and Minka, 2018). In the present study, the significant amounts of β-sheet structure in the Bambara groundnut protein may therefore explain, in part, its low solubility (Section 5.3.1). Additionally, no statistical difference ($p>0.05$) was detected in the composition of protein secondary structure amongst the Bambara groundnut genotypes. This corroborates previously discussed results (Section 5.3.1) where no genotypic variation was observed in protein solubility. On the other hand, Arise *et al.* (2017) suggested a possible association between protein secondary structure and its thermal stability. In this study, however, the secondary structure composition does not correspond to the DSC data reported in Section 5.3.2, suggesting that other factors, such as the protein tertiary structure, are more important in defining the thermal properties of Bambara groundnut flour.

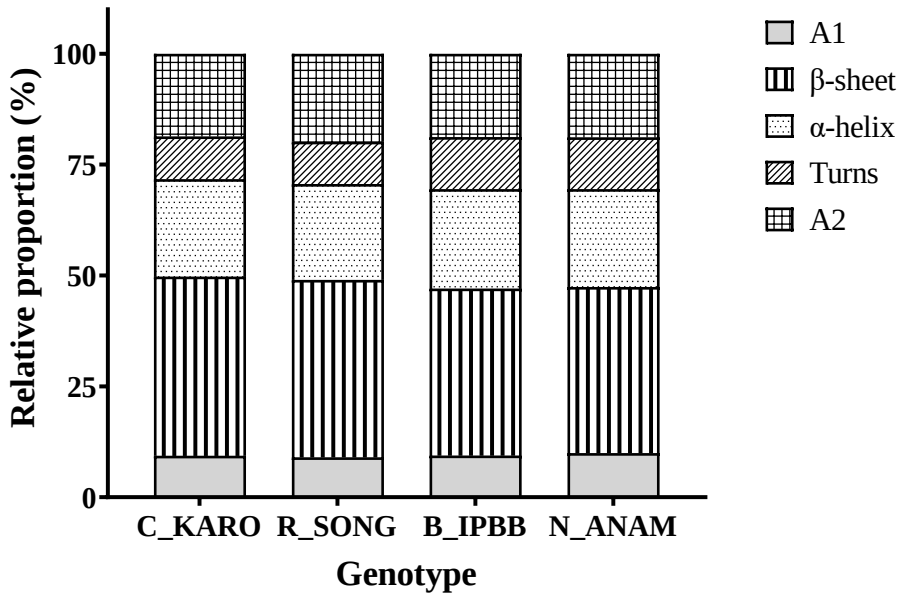

Figure 23 Relative percentage of protein secondary structures of flour of four Bambara groundnut genotypes.

5.3.4.3 AIR

The functional groups of interest for AIR lie between 1800 and 900 cm^{-1} of the spectrum. The spectra obtained for the Bambara groundnut AIR are shown in Figure 24. Overall, all samples showed a similar spectral profile, exhibiting major peaks at around 1745, 1630, 1514, 1230, 1152 and 1015 cm^{-1}. The peaks observed in the spectra of Bambara groundnut AIRs are in good agreement with the characteristic bands found in other plant cell wall materials (Table 11).

Table 11 Frequencies and assignments of infrared absorption bands of cell wall components based on the literature[a] (Synytsya *et al.*, 2003; Szymanska-Chargot and Zdunek, 2013; Chang *et al.*, 2014; Canteri *et al.*, 2019), in comparison to those obtained from the present experiment[b].

Frequency (cm^{-1})		Peak assignment/ description
Literature[a]	Experiment[b]	
1754-1740	1745	Carbonyl group (C=O) stretching of alkyl ester (pectin)
1653-1643	1642	Amide I (C=O stretching vibration)
1630-1600	1630	Carboxylate group (COO^-) stretching of pectin
1520-1500	1514; 1530	Phenolic backbone or alkene group (C=C) aromatic symmetrical stretching of lignin
1462-1444	1452	CH_2 bending of cellulose, lignin or CH_3 bend of pectin methyl group
1410-1400	1412	COO^- symmetric stretching of pectin ester group
1371-1370	1370	Symmetric CH_3 bending of xyloglucan, cellulose, and pectin methyl group
1335-1320	1340	CH deformation of ring vibration of polysaccharides/ pectin/ cellulose
1317-1312	1315	CH symmetric bending of xyloglucan and cellulose
1268-1230	1230	C-O stretching of pectin/ lignin
1160-1130	1152	O-C-O asymmetric stretching (glycosidic link of cellulose/ pectin/ xyloglucan)
1030-1000	1015	C-O stretching and C-C stretching (cellulose or pectin)

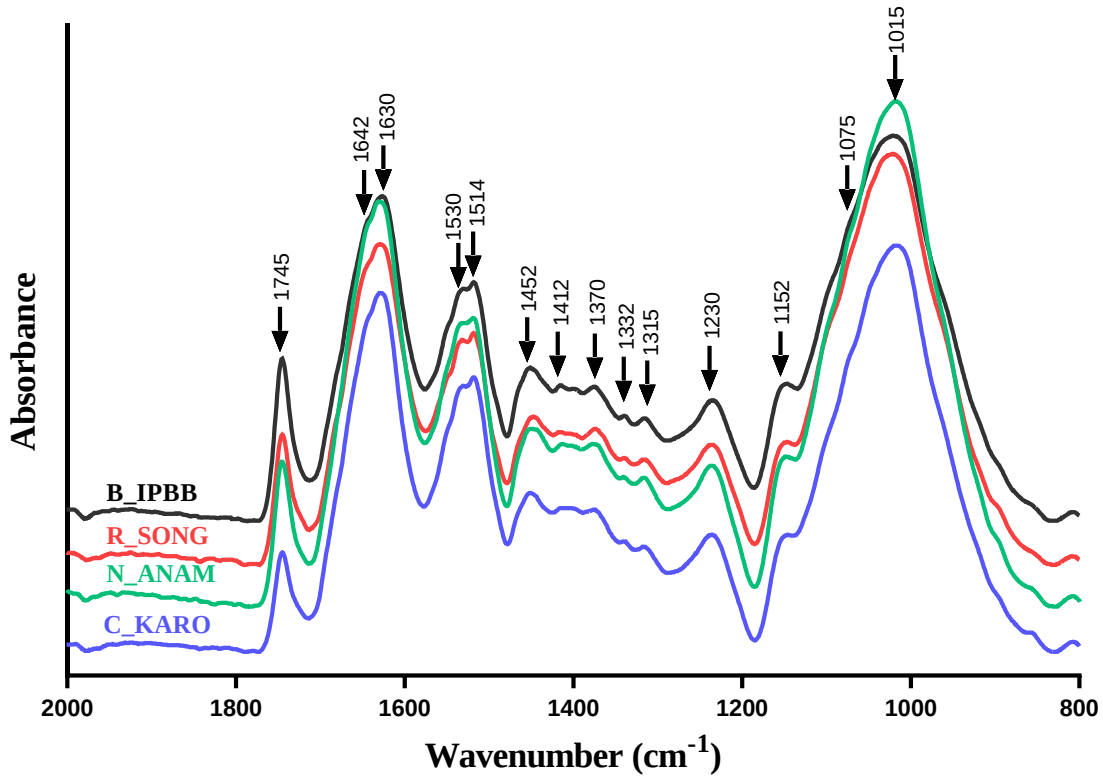

Figure 24 ATR-FTIR spectra of AIR extracted from four Bambara groundnut genotypes.

Two important peaks for AIR components are seen at about 1745 cm^{-1}, arising from the C=O stretching of alkyl ester, most likely due to esterified pectin, and around 1630 cm^{-1}, due to the COO^- stretching of non-esterified pectin. The ratio of these band intensities can therefore be used for calculation of pectin DoM (Kyomugasho *et al.*, 2015), which has been discussed in Section 5.3.3.

The shoulder at 1642 cm^{-1} may be assigned to the C=O (amide I) stretching vibration. The occurrence of the amide band in the AIRs, despite at a smaller amplitude when compared with the flours, suggests that the protein associated with cell walls, possibly as structural protein, had not been completely hydrolysed by protease during the extraction of AIR (Srisuma *et al.*, 1991).

The spectra for all samples exhibited a doublet at 1530 and 1514 cm^{-1}, which, together with the strong absorption at around 1630 cm^{-1}, suggests the presence of aromatics in the AIRs

(Coates, 2006), possibly due to phenolic compounds. The occurrence of a weak absorption at around 3010 cm^{-1} (not shown) further supports this diagnosis (Coates, 2006). These phenolic compounds, such as ferulic and ρ-coumaric acids, may cross-link to pectic polysaccharides, thereby promoting thermal stability of the cell wall structure (Garcia *et al.*, 1998; Mubaiwa *et al.*, 2019). Lignin, as an aromatic compound, may also be contributing to the peak at 1514 cm^{-1}. In addition, the bands at 1452 and 1230cm^{-1} are associated to lignin. It has been suggested that during ageing process, degradation of protein produces aromatic amino acids, which, as a precursor of lignin, could engage in cell lignification process and thus enhancing the mechanical strength of cell wall (Hincks and Stanley, 1987). According to Maurer *et al.* (2004), the area under the peak of the region 1708-1581 cm^{-1} is indicative of the concentration of phenolic compounds. However, as with the findings by the authors, the calculated peak areas did not differ ($p>0.05$) with genotypes (data not shown).

In the fingerprint spectral region (bands between 1500-800 cm^{-1}), a series of weak bands appeared in region 1450-1200 cm^{-1}, which could be related to polysaccharides such as pectin, xyloglucan, cellulose, and lignin. The shoulder at around 1075 cm^{-1} may be attributed to the presence of galactose unit in the form of rhamnogalacturonan and β-galactan (Kacuráková *et al.*, 2000). The most significant signal for the Bambara groundnut AIRs centred at around 1015cm^{-1}, which corresponds to the stretching vibrations of C-O and C-C groups. FTIR spectra of the cell wall materials of some vegetables also showed band maxima at around 1019 cm^{-1}, which the authors attributed to pectic polysaccharides (Szymanska-Chargot and Zdunek, 2013). Similarly, Kacuráková *et al.* (2000) reported the maximum band absorption for pectin was at 1017 cm^{-1}. Additionally, the maximum absorbance bands of polysaccharides galactan, arabinan, xyloglucan, glucan and glucomannan are located around 1050-1010 cm^{-1} (Kacuráková *et al.*, 2000), and therefore may also be contributing to the strong intensity of the band between 1100-950 cm^{-1}. This finding is in agreement with that of previous studies which reported that the major

constituents of neutral sugar in cell walls of common bean (Shiga and Lajolo, 2006; Yi *et al.*, 2016) and lentil (Bhatty, 1990) were glucose, xylose (in the form of xylogalacturonan and xyloglucan), arabinose and galactose. The spectral result, however, would require confirmation by quantification of pectin composition in future studies.

5.4 Conclusion

Chemical properties of pulses are one of the primary factors determining their cooking quality. Pectin, protein and starch are among the components often associated with the HTC phenomenon in legumes. In the present study, protein content, solubility, and secondary structure could not explain the differences in cooking times between Bambara groundnut genotypes. In addition, neither the pectin content nor the DoM were critical to the cooking time. The molecular structures of flour and cell wall materials were also similar between HTC and ETC genotypes. Rather, the abundance of tightly bound pectin and low levels of water-soluble pectin appeared to be the dominant factor imparting resistance to cell wall separation during cooking, causing delayed softening in HTC genotypes. The significantly ($p<0.05$) higher T_{peak} and ΔH exhibited by the HTC genotypes suggest that these genotypes contain starch and proteins with greater thermal stability, which may also have implications for prolonged cooking time. Despite these findings, it remains unclear whether the compositional and functionality differences between the ETC and HTC genotypes were due to intrinsic factor (genotypes), or whether they were a consequence of the extended storage period. Thus, a comprehensive investigation is required to evaluate the role of inherent compositional factors, as well as the effects of storage period and conditions on the chemical and thermal properties of Bambara groundnut to elucidate the hardening mechanism of the seed.

Chapter 6: Enhancing the hydration and cooking characteristics of Bambara groundnut with salt solution

6.1 Introduction

Various processing technologies, such as micronisation (Ogundele and Emmambux, 2018) gamma-irradiation (Falade and Adebiyi, 2015) and microwaving (Oyeyinka *et al.*, 2020), have been studied as potential options to resolve the hard-to-cook phenomenon in Bambara groundnut. Whilst these processes have shown promising results in reducing the cooking time of Bambara groundnut, they require sophisticated equipment that may not be available in impoverished regions. Another practical drawback is the additional energy cost to run the equipment in resource-limited areas.

Pre-soaking pulses in salt solutions has been proven to be a simple, effective and low-cost means to shorten their cooking times (Al-Nouri and Siddiqi, 1982; de León, Elías and Bressani, 1992). In the sub-Saharan regions, alkaline salt known as *kanwa* (Na_2CO_3·$NaHCO_3$·xH2O + impurities) is traditionally used to tenderise the beans (Mubaiwa *et al.*, 2017). However, inappropriate use of alkaline salts can have detrimental effects on the sensory quality of beans, such as causing unpleasant flavours or unacceptable colour changes (Al-Nouri and Siddiqi, 1982). Therefore, the composition and concentration of salt in the soaking solutions are critical parameters to ensure consumer acceptability.

The mixed salt solution developed by Rockland *et al.* (1979), containing 2% NaCl, 1% $Na_2P_3O_5$, 0.75% $NaHCO_3$ and 0.25% Na_2CO_3, has been shown to effectively reduce the cooking time of various pulses, e.g. winged bean, soybean, chickpea, common bean, lima bean and cowpea. Some of these salts are commonly used in household food preparation, indicating the practicality of this application. For instance, sodium chloride (NaCl), or table salt, is an

indispensable flavour enhancer; sodium bicarbonate ($NaHCO_3$) as a leavening agent; while sodium carbonate (Na_2CO_3), a main ingredient in *kansui*, is an essential ingredient in the production of yellow noodles which is popular in South-East Asia (Lai *et al.*, 2002).

The mechanisms through which salt solution induces softness may be related to several biochemical effects exerted by salts. First, it has been postulated that the favourable binding of monovalent cations (e.g. Na^+) to pectic substances leads to the displacement of divalent cations (e.g. Ca^{2+}) from insoluble pectate, thus enhancing pectin solubilisation (Varriano-Martson and De Omana, 1979). This, in turn, weakens the intercellular adhesion and promotes cell separation during cooking. Secondly, anions (e.g. CO_3^{2-} and PO_4^{3-}) have been suggested to participate as chelating agents for divalent cations of insoluble pectate, thus increasing the softening rate (Varriano-Martson and De Omana, 1979). Garcia-Vela *et al.* (1991), however, disagreed with the chelation and ion-exchange mechanisms. The authors hypothesised that the effect of salt in reducing cooking time was through the action of anions that destabilises the storage proteins, thereby rendering them more heat labile. Lastly, investigation by Mubaiwa *et al.* (2019) has provided evidence to support the link between enhanced solubilisation of bound phenolic compounds and shorter cooking time of Bambara groundnut cooked in alkaline salt solution.

Whilst it is known that the use of salt solutions has a tenderising effect on pulses, its influence on the processing characteristics of Bambara groundnut is lacking in the literature. This study was divided into two experiments. The first experiment was undertaken to optimise the concentration of sodium salts in soaking solution. Hydration capacity, cookability, and colour of cooked seeds were the attributes used to monitor the optimisation. The objective of the second experiment was to evaluate the roles of the optimised salt solution in influencing the hydration and cooking characteristics of Bambara groundnut.

6.2 Materials and methods

6.2.1 Materials

Cream-seeded genotype C_NAV4 (Section 3.1) was selected for this study due to the popularity of cream-coloured seeds for domestic consumption (Abu and Buah, 2011) and the drought- and heat-tolerant and high-yielding properties of this genotype (Berchie *et al.*, 2012).

6.2.2 Part I: Optimisation of salt concentrations in soaking solution

6.2.2.1 Experimental design

A central composite rotatable design (CCRD) with three factors and five levels (Table 12) was used to evaluate the optimised salt concentration for soaking solution. Concentrations of NaCl, $NaHCO_3$ and Na_2CO_3 in soaking solution were the three independent variables; whereas hydration capacity (HC), colour difference of cooked seeds (ΔE*), and percentage of cooked seeds (%C) were the dependent variables studied. The type of salt and their concentration range were selected based on the formula by Rockland *et al.* (1979) (2% NaCl, 1% $Na_2P_3O_5$, 0.75% $NaHCO_3$ and 0.25% Na_2CO_3). To ensure that the salt solution is applicable at household level, only NaCl, $NaHCO_3$ and Na_2CO_3 were selected as these salts are commonly available for domestic cooking.

Table 12 Independent variables and levels used for CCRD.

Factors	Code	Variable level				
		-1.68	-1	0	+1	+1.68
NaCl concentration (g/100 mL)	X_1	0.00	0.51	1.25	2.00	2.51
$NaHCO_3$ concentration (g/100 mL)	X_2	0.00	0.19	0.47	0.75	0.94
Na_2CO_3 concentration (g/100 mL)	X_3	0.000	0.064	0.158	0.251	0.315

The experiment featured duplicate determination for axial and factorial points and five replications for the centre point, summing to 33 runs in total (Table 13). Design-Expert®

(Version 13, Stat-Ease Inc, USA) was used to generate three-dimensional plots of the response surface model and to determine the optimised salt concentration. Optimum conditions were obtained by minimising colour changes and maximising hydration capacity and % cooked seeds to achieve the highest desirability of 1. The importance of colour change and hydration capacity was set at ++++, whereas importance of percentage of cooked seeds was set at +++++.

6.2.2.2 Soaking and cooking procedures

Fifty seeds were weighed and soaked in various soaking solutions (Table 13) at ratio of 1:5 (w/w) for 16 h at room temperature. To cook the seeds, the soaked seeds were drained and added to a beaker containing distilled water (1:5 w/w) preheated to 98°C in a water bath. The beaker was covered with aluminium foil throughout the cooking process. The seeds were cooked for 60 min, after which they were removed from cooking water and let cool to room temperature.

6.2.2.3 Hydration capacity

After the soaking time, the seeds were removed from the soaking liquid, blotted dry and weighed. The hydration capacity (HC) was expressed as the % weight gained per gram of seeds after 16 h.

$$HC\ (\%) = \frac{Soaked\ seed\ weight\ (g) - dry\ seed\ weight\ (g)}{Dry\ seed\ weight\ (g)} \times 100\% \qquad (16)$$

6.2.2.4 Colour difference of cooked seeds

The colour of cooked seeds was determined within 2 h of cooking using a colourimeter as with Section 3.2.1. The average of 20 measurements was used to determine the colour difference against the control (cooked seeds pre-soaked in distilled water), using the equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (17)$$

where ΔL*, Δa*, and Δb* are the differences in L*, a* and b* between salt solution-soaked seeds and distilled water-soaked seeds, respectively.

6.2.2.5 Percentage of cooked seeds (Cookability)

Cooking status of the seeds was determined by finger-pressing method and white-core method as described in Section 4.2.13. The number of cooked seeds was counted and cookability was expressed as the percentage of cooked seeds after cooking for 1 h.

6.2.3 Part II: Effects of soaking solution on the imbibition and cooking characteristics of Bambara groundnut

Following the selection of the optimised salt solution, the effects of salt solution on rehydration characteristics, cooking quality and morphology of seeds were assessed.

6.2.3.1 Soaking procedure

Seed samples were weighed, then soaked in 1:5 ratio (w/w) of distilled water (DW) or optimised salt solution (SS; 0.25% $NaHCO_3$ + 0.14% Na_2CO_3; pH = 9.85; ionic strength = 0.13 M) at room temperature until equilibrium moisture content was reached.

6.2.3.2 Rehydration kinetics

Rehydration kinetics were determined as described in Section 4.2.10, taking into consideration the initial moisture content of raw seeds. Since the hydration curves showed a sigmoidal shape, a sigmoidal regression equation was used to model the rehydration kinetic (Equation 13).

6.2.3.3 Soluble solid losses during soaking

Soaking solution (2 mL) was sampled every 4 h for 32 h. Soluble solid losses (%) in soaking liquid were determined according to Section 3.2.3. The refractometer was standardised with distilled water and salt solution before determination of solid loss in the respective soaking solution.

6.2.3.4 pH

The pH of the soaking liquid was determined at specific time intervals using pH meter (Section 3.2.4).

6.2.3.5 Thermal properties

Dry (unsoaked) and soaked seeds were subjected to thermal analysis. Soaked seeds were kept frozen at -80°C before freezing drying (Alpha 1-4 LD plus, Christ, Germany). Freeze dried seeds were ground using a mortar and pestle to pass through a 0.25 mm sieve. Thermal analysis was performed as with Section 3.2.6.

6.2.3.6 Pectin solubility

The AIR was extracted according to Section 5.2.4.1. Pectin solubility was determined using the colourimetric assay described in Section 5.2.4.2 and 5.2.4.3. Galacturonic acid (GalA) was used as standard (1-40 µg GalA/mL), and the standard curve was linear (R^2 = 0.9969).

6.2.3.7 Cooking procedure

To minimise the effect of moisture levels on the cookability of seeds, the seeds were pre-soaked for 24 h to ensure that the moisture contents between distilled water- and salt solution-soaked seeds were not significantly different ($p>0.05$). The soaking liquid was discarded, and the seeds were placed in a covered beaker containing fresh distilled water preheated to 98°C. The seeds were cooked in a water bath for 100 min. The beakers were covered with aluminium foil throughout the cooking period. Raw, unsoaked seeds were also subjected to cooking to assess the effect of soaking process.

6.2.3.8 Water uptake during cooking

The seeds were removed from the cooking solution every 20 min, quickly blotted dry and weighed. The seeds were then immediately replaced to the beaker to continue cooking process. Water uptake was calculated as the percent of water absorbed per gram of precooked seeds.

6.2.3.9 Soluble solid losses during cooking

Cooking solution (2 mL) was sampled every 20 min. Soluble solid losses (%) in cooking liquid were determined according to Section 3.2.3.

6.2.3.10 Colour evaluation

The colour of cooked seeds was determined using a colourimeter as described in Section 3.2.1.

The chroma (C*) and hue angle (h°) was calculated as:

$$C^* = (a^{*2} + b^{*2})^{\frac{1}{2}} \quad (18)$$

$$h° = arctan(\frac{b^*}{a^*}) \quad (19)$$

6.2.3.11 Cooking profile and kinetics

Seeds were withdrawn from the cooking solution every 20 min to determine the doneness of cooking. Cooking status of the seeds was determined by finger-pressing method and white-core method as described in Section 4.2.13. The accumulated number of cooked seeds was counted and plotted against time to visualise the cooking curve. The cooking kinetics was then characterised using a two-parameter sigmoidal model:

$$C(t) = \frac{100}{1 + exp[-k_C \cdot (t - \tau_C)]} \quad (20)$$

where C(t) is the percentage of cooked seeds at time t, k_c is the cooking kinetic constant, and τ_c is the time needed to cook 50% of seeds.

6.2.3.12 Grain morphology

Seeds were withdrawn from the water bath every 20 min, frozen in a -80°C freezer, and then freeze dried. Lyophilised seeds were transversely fractured using a scalpel blade. The SEM viewing process was performed according to Section 3.2.5.

6.2.4 Statistical analysis

Independent-samples t-test was used to compare means when there were two independent variables (DW-soaked and SS-soaked samples). When there were three independent variables (unsoaked, DW-soaked, and SS-soaked seeds), data was analysed with one-way ANOVA and means were compared by Tukey *post hoc* test if significant difference ($p<0.05$) was found.

6.3 Results and discussion

6.3.1 Evaluation of the effect of salt concentrations on dependent variables

6.3.1.1 Hydration capacity (HC)

The HC of Bambara groundnut soaked in different salt solution varied from 55.93 (1.25% NaCl + 0.47% $NaHCO_3$ + 0.158% Na_2CO_3) to 76.46% (0.51% NaCl + 0.75% $NaHCO_3$ + 0.064% Na_2CO_3) (Table 13). A significant (p=0.0001) linear model was obtained for the HC with a non-significant lack-of-fit (p>0.05), indicating that the experimental data fitted well to the model. The determination coefficient was relatively low at R^2=0.509, indicating that the model could only explain 50.9% of variations in HC. Such low R^2 could be attributed to the wide variations in water imbibition rate exhibited by individual seeds.

The equation established to predict the HC was: $HC = 64.27 - 3.84X_1 + 0.0006X_2 + 1.18X_3$, where X_1, X_2 and X_3 represent the concentrations of NaCl, $NaHCO_3$ and Na_2CO_3, respectively. By comparing the factor of coefficients of the equation, the concentration of NaCl had a relatively greater effect on the HC than that of Na_2CO_3, whereas the concentration of $NaHCO_3$ had negligible impact. This is in line with the results of analysis of variance (ANOVA), which revealed that the NaCl concentration had a significant influence (p<0.0001) on the HC. The graphic illustration of the response surface is shown in Figure 25.

Based on the model, the HC increases with decreasing concentration of NaCl and increasing concentrations of $NaHCO_3$ and Na_2CO_3. The decrease in water uptake at higher NaCl concentrations might be due to the salting-out phenomenon of protein (Kudre, Benjakul and Kishimura, 2013), which occurs when there are high concentrations of salt ions in the solution (high ionic strength). These salt ions compete with charged amino acids for interaction with water molecules, thereby reducing the amount of water bound to the protein. Moreover, accumulation of salt ions around the charged residues on protein surface may also reduce its surface charges, thus preventing the polar groups from binding to water (Kudre, Benjakul and Kishimura, 2013).

On the other hand, alkaline salts appeared to exert positive effect on the HC of Bambara groundnut. The enhanced hydration could be associated with the higher pH levels conferred by CO_3^{2-} (Garcia-Vela and Stanley, 1989b). Bambara groundnut protein has an isoelectric point between pH 4-5 (Kudre, Benjakul and Kishimura, 2013). As pH rises, the increase in the tendency of protein to ionise causes an increase in net charge on the protein surface. This promotes protein-solvent interaction, thereby increasing hydration and solubility of protein molecules. In this study, the effect of pH was probably greater than the effect of ionic strength, thus resulting in an overall positive effect on the hydration of the seeds.

Starch and non-starch polysaccharides also contains hydrophilic regions that may interact with salt ions and affect water absorption of the seeds (Sirivongpaisal, 2008). For instance, alkaline pHs have been shown to increase the water solubility of pea starch, while the addition of NaCl had the opposite effect (Choi, Patel and Han, 2016).

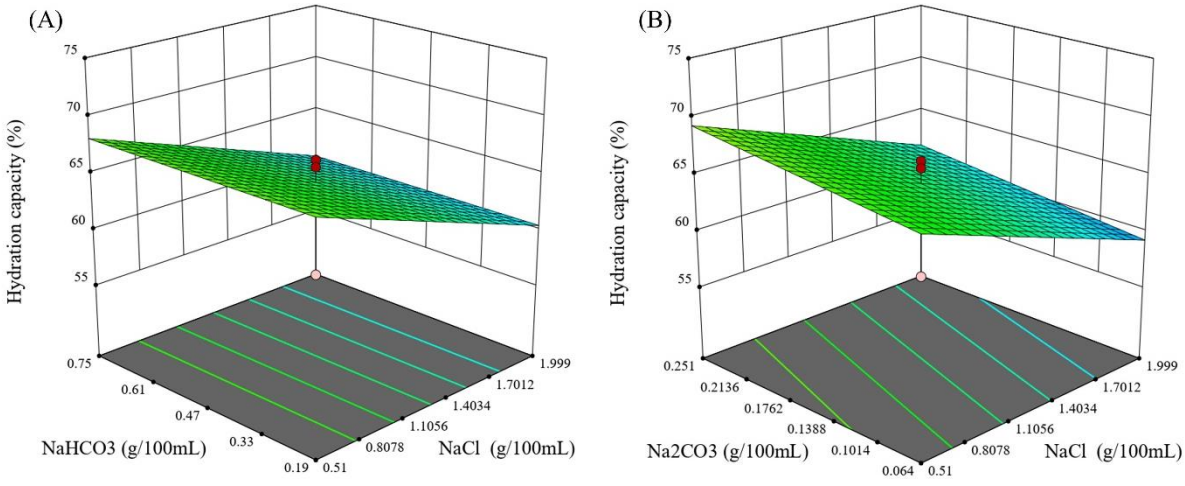

Figure 25 Response surface plots of hydration capacity as a function of (A) NaCl and $NaHCO_3$ concentrations and (B) NaCl and Na_2CO_3 concentrations.

6.3.1.2 Percentage of cooked seeds (%C)

The % of cooked seeds ranged from 56 (2% NaCl + 0.19% $NaHCO_3$ + 0.064% Na_2CO_3) to 82% (0.51% NaCl + 0.19% $NaHCO_3$ + 0.251% Na_2CO_3) (Table 13). As with HC, the cookability of seed showed a linear response on the concentrations of NaCl, $NaHCO_3$ and Na_2CO_3 with R^2=0.502 ($p<0.0001$) and a non-significant lack-of-fit ($p>0.05$). The low R^2 was likely due to the wide intra-sample variability in cooking time among Bambara groundnut seeds, which was also observed in earlier experiment (Section 4.3.4.1). Such variability is believed to be an inherent trait, and its effect could be enhanced by ageing process (Chen *et al.*, 2021). The % of cooked seeds at different salt concentrations can be predicted from the regression equation: $\%C = 71.39 - 3.35X_1 + 2.65X_2 + 2.32X_3$, where X_1, X_2 and X_3 represent the concentrations of NaCl, $NaHCO_3$ and Na_2CO_3, respectively. The ANOVA result indicates strong dependence of seed cookability on all three independent variables ($p<0.05$).

As depicted in Figure 26, the highest %C occurred at the lowest concentration of NaCl and the highest concentrations of $NaHCO_3$ and Na_2CO_3. Since all three salts contained the Na^+ ions, the result suggests that it was not the cations, but rather the anions (CO_3^{2-}), that played a key role in promoting the cookability of Bambara groundnut. The involvement of monovalent sodium cations in bean softening has previously been associated with its tendency to displace divalent cations bound to pectate and thus promoting pectin solubilisation, as well as its ability to positively affect the heat transfer behaviour of the bean (de León, Elías and Bressani, 1992). On the other hand, anions may enhance texture degradation during cooking by destabilising protein (Garcia-Vela, del Valle and Stanley, 1991), and by chelating divalent cations to facilitate cell wall dissociation (Varriano-Martson and De Omana, 1979). Similar to this study, Garcia-Vela *et al.* (1991) reported that CO_3^{2-} was highly effective in tenderising the bean during cooking and concluded that the type of anion present in soaking solution was a major factor affecting the softening rate during cooking. The authors, however, also found an enhanced softening effect

with increasing levels of NaCl from 0.1 to 1.0 M ionic strength, which was not in agreement with the results of this study. The conflicting results may be due to different type of legume used, which may have different responses to the NaCl solution due to compositional differences. In the current study, increasing concentrations of NaCl resulted in lower hydration levels of seeds before cooking (Section 6.3.1.1) and this may partially explain the lower % of cooked seeds.

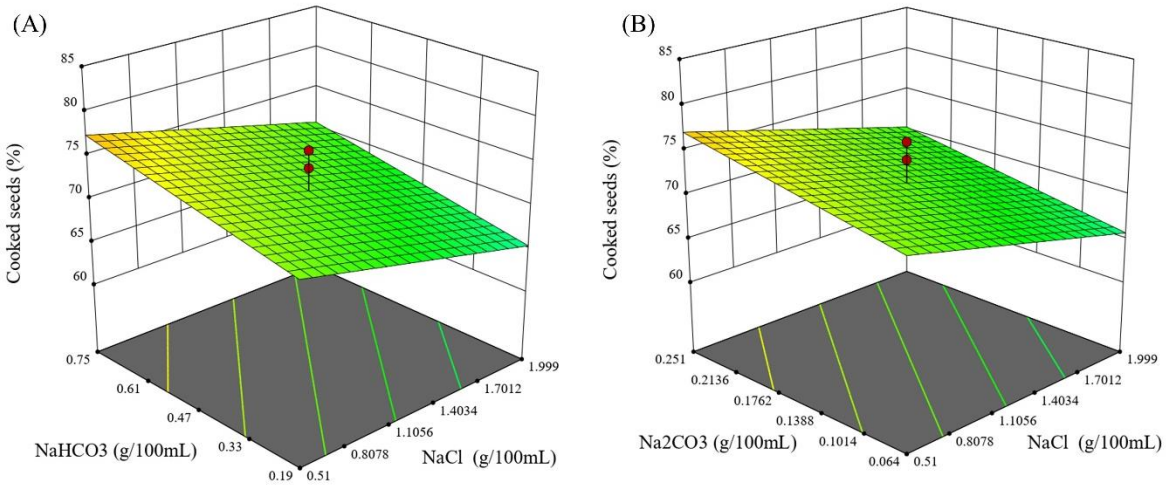

Figure 26 Response surface plots of percentage of cooked seeds as a function of (A) NaCl and $NaHCO_3$ concentrations and (B) NaCl and Na_2CO_3 concentrations.

6.3.1.3 Colour change

The colour difference in cooked seeds between seeds soaked in distilled water (control) and various salt solutions was measured as colour change (ΔE*), which ranged between 3.523 (1.25% NaCl + 0.158% Na_2CO_3) and 9.746 (0.51% NaCl + 0.75% $NaHCO_3$ + 0.251% Na_2CO_3) (Table 13). A greater extent of colour change is undesirable from consumers point of view. The colour change of cooked Bambara groundnut exhibited a significant ($p<0.0001$) linear regression model ($R^2=0.712$; lack-of-fit $p>0.05$) with respect to salt concentration. The regression equation obtained to predict the % of cooked seeds was $\Delta E^* = 6.19 + 0.2519X_1 + 0.8543X_2 + 1.14X_3$, where X_1, X_2 and X_3 represent the concentrations of NaCl, $NaHCO_3$ and Na_2CO_3, respectively

(Figure 27). The ANOVA shows that all types of salt had a negative impact on the colour change of cooked seeds, in which the $NaHCO_3$ and Na_2CO_3 resulted in a more pronounced ($p<0.0001$) colour change in comparison to NaCl ($p>0.05$).

Colour change in cooked beans with increasing levels of $NaHCO_3$ and Na_2CO_3 generally trended towards a lower L* value (lightness), and a greater a* (redness) value on CIELAB scale (Appendix 4), indicating browning of cooked bean (Arntfield *et al.*, 1997). Addition of alkaline salts in soaking or cooking solutions tended to produce darker coloured seeds after cooking, as demonstrated in cowpea (Uzogara, Morton and Daniel, 1988) and broad bean (Al-Nouri and Siddiqi, 1982). The darkening of seeds may be caused by the formation of metal complexes between metal ions and pigments of the seeds, or by the oxidation of leached pigments (Uzogara, Morton and Daniel, 1988). Pearson correlation analysis indicates that the ΔE* value was strongly and significantly ($p<0.01$) correlated with L* ($r=-0.997$) and a* ($r=0.776$), reflecting that $NaHCO_3$ and Na_2CO_3 induced colour changes mainly by decreasing colour lightness and increasing redness of the seeds.

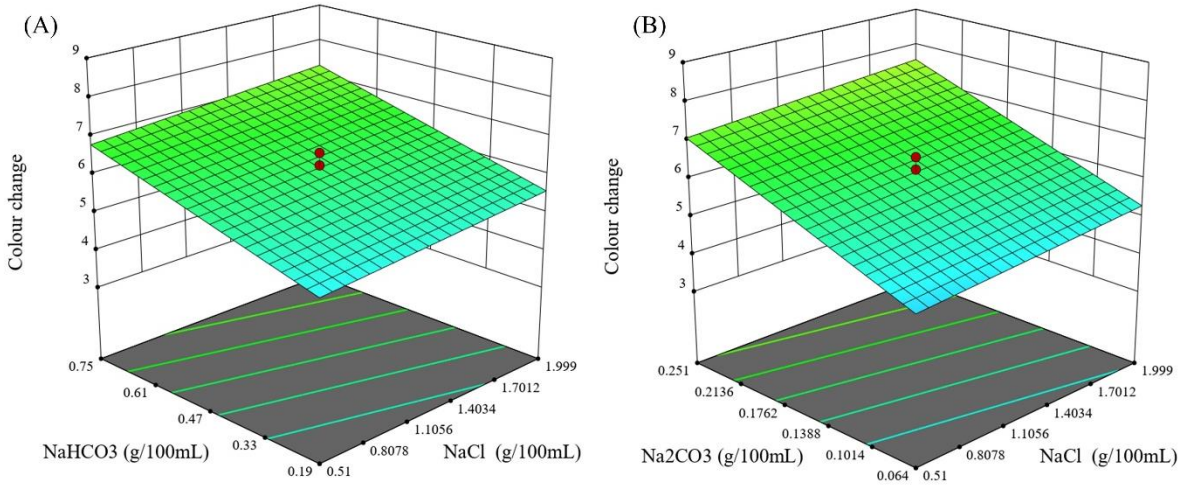

Figure 27 Response surface plots of percentage of cooked seeds as a function of (A) NaCl and $NaHCO_3$ concentrations and (B) NaCl and Na_2CO_3 concentrations.

Table 13 Design matrix and variable combinations of salt concentration in experimental runs, and responses of dependent variables hydration capacity (HC); % of cooked seeds (%C) and colour changes (ΔE*) of the cooked bean.

Std order	Run order	Independent variables			Dependent variables		
		NaCl (g/ 100mL)	$NaHCO_3$ (g/ 100mL)	Na_2CO_3 (g/ 100mL)	HC (%)	%C (%)	ΔE*
11	1	2.00	0.19	0.251	57.39	60	6.350
13	2	0.51	0.75	0.251	68.44	78	8.038
21	3	1.25	0.00	0.158	65.91	68	3.836
24	4	1.25	0.94	0.158	61.02	72	7.105
7	5	2.00	0.75	0.064	56.79	64	5.682
3	6	2.00	0.19	0.064	59.00	56	5.457
23	7	1.25	0.94	0.158	61.18	78	7.571
30	8	1.25	0.47	0.158	62.20	76	6.262
31	9	1.25	0.47	0.158	66.24	74	5.720
12	10	2.00	0.19	0.251	60.75	66	7.161
18	11	0.00	0.47	0.158	75.36	70	5.754
9	12	0.51	0.19	0.251	64.93	76	7.682
19	13	2.51	0.47	0.158	59.76	64	7.200
8	14	2.00	0.75	0.064	57.86	64	6.591
10	15	0.51	0.19	0.251	73.33	82	5.149
27	16	1.25	0.47	0.315	69.43	78	8.401
14	17	0.51	0.75	0.251	69.97	78	9.746
16	18	2.00	0.75	0.251	68.02	82	8.345
4	19	2.00	0.19	0.064	62.94	66	4.482
32	20	1.25	0.47	0.158	63.23	76	5.947
5	21	0.51	0.75	0.064	61.52	74	3.785
17	22	0.00	0.47	0.158	69.79	72	6.147
33	23	1.25	0.47	0.158	65.61	76	6.586
15	24	2.00	0.75	0.251	63.59	72	8.426
28	25	1.25	0.47	0.315	63.47	68	8.174
29	26	1.25	0.47	0.158	55.93	74	5.415
1	27	0.51	0.19	0.064	64.13	76	4.593
25	28	1.25	0.47	0.000	60.07	68	4.662
20	29	2.51	0.47	0.158	60.17	70	5.753
6	30	0.51	0.75	0.064	76.46	78	4.840
2	31	0.51	0.19	0.064	70.09	66	3.551
22	32	1.25	0.00	0.158	62.28	64	3.523
26	33	1.25	0.47	0.000	64.15	70	6.474

6.3.2 Optimisation of soaking solution

Based on the findings, numerical optimisation was performed to establish the optimal soaking solution to maximise hydration capacity and cookability of seeds while restricting colour change after cooking. The optimal soaking solution obtained was a solution containing 0.25% $NaHCO_3$ and 0.144% Na_2CO_3 with a desirability of 0.732. The desirability value indicates that when the seeds are soaked in this solution, 73.2% of the desirable outcomes (maximised HC and cookability; minimised ΔE*) will be achieved. Interestingly, NaCl was omitted from the optimised solution, probably because its addition did not offer any advantages in improving the hydration and cooking rates of the bean.

Using this soaking solution, Bambara groundnut was expected to achieve a HC value of 70%, with 74.62% of cooked seeds and a ΔE* value of 4.933. To validate the model, separate experiments were performed to compare the experimental data and model predicted data. The values of HC, %C and ΔE* obtained were 67.98%, 68% and 4.975, respectively. The relatively small deviations indicate the adequacy of this model in optimisation of soaking solution.

The influence of salt solution on the hydration and cooking behaviour of pulses can be cultivar-dependent, as observed in chickpea (Avola and Patanè, 2010) and common bean (Kinyanjui *et al.*, 2015). Therefore, it is acknowledged that the optimum salt concentration obtained from this experiment may not necessarily improve the hydration rate or the cookability of other Bambara groundnut varieties. Nonetheless, this optimised salt solution would serve as a variable to enable comparisons between the effects of different pre-treatments.

6.3.3 Effect of soaking medium on soaking behaviour

6.3.3.1 Rehydration kinetics

The water uptake curves of Bambara groundnut soaked in distilled water (DW) and the optimised salt solution (SS) exhibited a sigmoidal pattern, which was characterised by an initial lag phase, an intermediary exponential phase, and a final plateau phase (Figure 28). The experimental hydration data was modelled using a sigmoidal equation (Equation 13; Kaptso *et al.*, 2008) to estimate three parameters: (i) equilibrium moisture content (M_{eq}), which is related to the plateau phase; (ii) rate constant of hydration (k), which corresponds to the slope of the exponential phase; and (iii) time required to attain half saturation (τ), which is related to lag phase time.

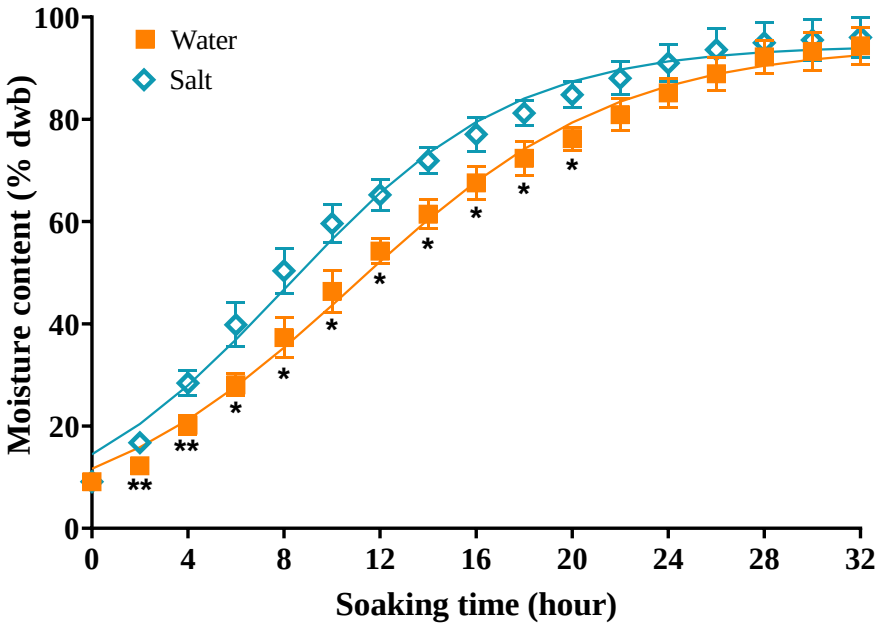

Figure 28 Hydration curves of Bambara groundnut soaked in different solution as a function of soaking time. Markers are means of experimental values (n=3); vertical bars represent standard deviation; lines denote model-predicted curves; * and ** indicate significant difference in moisture content between treatments at $p<0.05$ and $p<0.01$, respectively, based on student's t-test.

The estimated values of these parameters are shown in Table 14. Overall, the model showed good fit for the experimental data with high coefficients of determination (R^2>0.99) and low mean relative deviations (E<10%). Both samples showed a similar level of M_{eq}, indicating that the maximum capacity of seeds to absorb water was unaffected by soaking solution. On the contrary, the SS-soaked seeds showed a 20% higher k value and a 34% lower τ value, indicating a higher rate of water imbibition and a shorter lag phase for the SS-soaked seeds. This finding is supported by the hydration curves in Figure 28, which shows a systematically higher water uptake by SS-soaked seeds during the first 20 h of soaking. This is in contrast to previous studies which found an adverse effect of salt solution on the hydration behaviour of legume seeds (Hsu, Kim and Wilson, 1983; Del Valle, Stanley and Bourne, 1992). The authors attributed their observations to the increased solute concentration in the soaking solution, which, in turn, led to increased solution viscosity, lowered water activity, and decreased osmotic potential. The mechanisms involving solution viscosity and water activity, however, has been refuted recently by Vasquez *et al.* (2021). In fact, the use of alkaline salt solution has been reported to have variable effects on the imbibition behaviour of other legumes, depending on the concentration and the type of salt, as well as the pH of the soaking solution (Garcia-Vela and Stanley, 1989b; Ávila *et al.*, 2015).

Table 14 Estimated parameters of the sigmoidal model (Equation 13) on water absorption data.

Soaking solution	M_{eq} (% dwb)	k (h^{-1})	τ (h)	R^2	E (%)
Water	94.545 ± 1.523	0.181 ± 0.009	10.853 ± 0.313	0.995	5.75
Salt	94.519 ± 1.442	0.211 ± 0.013	8.113 ± 0.306	0.992	7.30

Values are means ± standard errors (n=3).

In the present study, the improvement in the soaking efficiency of seeds in SS could be partially attributed to the high pH environment created by SS (pH 9.86), which caused an increase in the exposure of negatively charged amino acids and, subsequently, a higher net surface charge of protein (Kudre, Benjakul and Kishimura, 2013; Oladele *et al.*, 2018). This may enhance the electrostatic response of protein with the surrounding water and promote hydrogen bonding, thereby increasing water absorption. Furthermore, a slightly higher ionic strength (0.13 M) in the SS favours protein unfolding due to increased repulsive forces, thereby enhancing hydrophilic interactions at the protein-solvent interface (Ngui *et al.*, 2021).

The shorter lag phase observed for SS-soaked seeds indicates a higher water imbibition rate at the early stages of soaking. Since the seed coat acts as a moisture barrier to retard initial water uptake of Bambara groundnut (Section 4.3.2.1), the reduced lag time may imply that the salt solution increased the water permeability of the seed coat. Vásquez *et al.* (2021) also reported a lower lag time in the water uptake of pigeon pea (*Cajanus cajan*) soaked in $NaHCO_3$ solution. The authors postulated that the alkaline solution lowered the glass transition temperature (T_g) of seed coat, possibly by modulating the composition or molecular interactions of the seed coat. A lower T_g therefore promotes the glass-to-rubber transition of seed coat and induces its permeability to water, consequently reducing the lag phase time.

6.3.3.2 Leaching during soaking

Figure 29 presents the solid loss and pH changes in the soaking media during soaking process. There was a rapid fall in the pH of distilled water during the first 4 h of soaking. The drastic decrease in pH could be caused by the acidic substances on the seeds' external surface, which composition remains unknown. In a separate experiment, the pH of DW was measured at 30 min interval. The results revealed an approximately pH 1 decrease in DW after 30 min of soaking, when water imbibition had not begun. At low moisture contents, the testa of Bambara groundnut is impermeable to water, and mass exchange occurs predominantly through the hilum (Section 4.3.2.1). The absence of water uptake therefore indicates a closed hilum. Hence, it can be inferred that leaching had not occurred during this period, confirming that leachate of seeds was unlikely to cause such drastic decrease in pH. In contrast to DW, the pH of the SS remained relatively constant during this period, probably because these acidic substances had been neutralised by excessive hydroxyl ions in the salt solution (Oladele *et al.*, 2018).

Overall, the pH of both soaking solutions decreased as soaking time increased. The decreasing trend may be due to the hydrolysis of protein, pectin or other organic acids that resulted in the liberation of H^+ which may then diffuse into soaking liquid (Varriano-Martson and De Omana, 1979). The pH drop was more pronounced in the SS during 12-28 h soaking, probably because the solution has lost its buffering capacity following the build-up of H^+ ions.

Soluble solids leached into SS were consistently higher ($p<0.05$) for SS-soaked samples than for DW-soaked samples throughout the soaking period, suggesting modified permeability of testa and/or cotyledon cell wall for the SS-soaked seeds. This observation corresponds to their respective hydration characteristics (Section 6.3.3.1). The addition of alkaline salt to the soaking solution may affect the composition of the leachate, which may, in turn, influence the nutritional quality of the cooked bean. For example, addition of carbonate salt in soaking solution has been shown to cause a significant reduction in heat-stable antinutrients particularly tannins, phytic acid

and oligosaccharides in other pulses (Vadivel and Pugalenthi, 2009; Devi *et al.*, 2018). Concomitant with the reduction of antinutrients, however, was an increase in leaching of essential nutrients such as B-vitamins (Prodanov, Sierra and Vidal-Valverde, 2004), total soluble sugars, and starch (Rehman, Salariya and Zafar, 2001), which can be undesirable from a nutritional standpoint. Protein is also more likely to leach out as its solubility increased with pH (Kudre, Benjakul and Kishimura, 2013). The overall impact of soaking in alkaline salt solution on nutritional quality of Bambara groundnut requires a comprehensive investigation and is beyond the scope of this study.

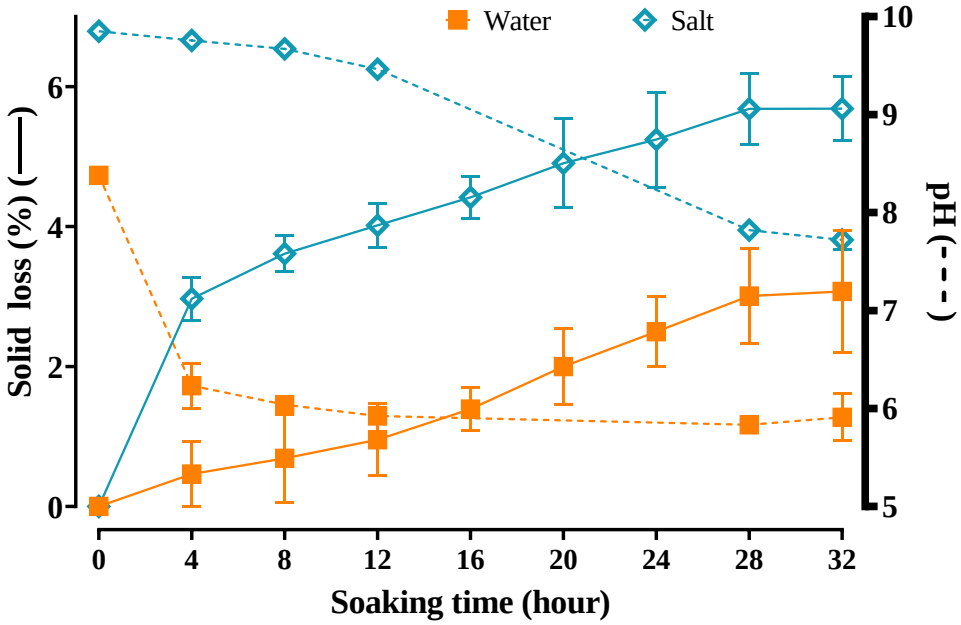

Figure 29 Solid loss (——) and pH (- - -) vs soaking time of Bambara groundnut soaked in different solutions. Markers are the averaged experimental data (n=3), vertical bars are the standard deviation.

6.3.4 Thermal and chemical changes after soaking

6.3.4.2 Differential scanning calorimetry (DSC)

The thermal properties of flours prepared from unsoaked, DW-soaked, and SS-soaked seeds are presented in Table 15. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and enthalpy change (ΔH) of unsoaked (control) sample were comparable to those found in other genotypes of Bambara groundnut (Section 5.3.2).

Soaking process significantly ($p<0.05$) reduced the T_o, indicating that thermal transitions commenced at a lower temperature for the soaked seeds. Contrarily, the T_p and T_c remained constant ($p>0.05$) irrespective of treatments, suggesting that the treatment did not greatly affect the overall thermal stability of the biopolymers. The increase in the range of thermal transition ($T_c - T_o$) for the soaked samples indicates a higher heterogeneity of thermal stability among the starch and protein components. A marked decrease in the magnitude of the endothermic peak among the soaked samples (Figure 30) led to a significant ($p<0.05$) decrease in ΔH. A lower ΔH suggests that the proteins might have undergone partial denaturation during the soaking process.

In the present study statistical analysis revealed non-significant ($p>0.05$) difference in the thermal properties between DW- and SS-soaked seeds. The addition of carbonate salt has been shown to lower the denaturation temperature and enthalpy of protein in black bean (Garcia-Vela, del Valle and Stanley, 1991; del Valle *et al.*, 1992), which the authors attributed to the ability of CO_3^{2-} ions to solubilise or destabilise protein structure. Conversely, alkali treatment using Na_2CO_3 has been reported to increase the thermal transition temperatures of various cereal starches, possibly due to stabilisation of starch granule structure by the Na^+ ions (Lai *et al.*, 2002). Hence, it is conceivable that the starch stabilising effect of Na^+ was offset by the protein destabilising effect of CO_3^{2-}, resulting in the net result of insignificant differences between the pre-soaked samples. However, due to the confounding starch gelatinisation and protein

denaturation endotherms, this postulation would require further investigation by studying the thermal properties of the starch and protein isolated from the Bambara groundnut.

Table 15 Thermal transition parameters of unsoaked, DW-soaked, and SS-soaked Bambara groundnut flours.

Treatment	Thermal transitions (°C)				Enthalpy, ΔH (J g^{-1})
	T_o	T_p	T_c	T_c - T_o	
Unsoaked	75.7 ± 0.12[a]	80.8 ± 0.26[a]	91.6 ± 0.64[a]	15.9 ± 0.53[a]	7.30 ± 0.21[a]
DW-soaked	73.9 ± 0.20[b]	80.6 ± 0.20[a]	90.8 ± 0.40[a]	16.9 ± 0.55[b]	6.43 ± 0.11[b]
SS-soaked	74.3 ± 0.21[b]	80.6 ± 0.15[a]	91.3 ± 0.25[a]	17.1 ± 0.42[b]	6.47 ± 0.15[b]

Values are means ± SD (n=3). Values with different superscript letters within a column are significantly different ($p<0.05$) with Tukey post hoc test.

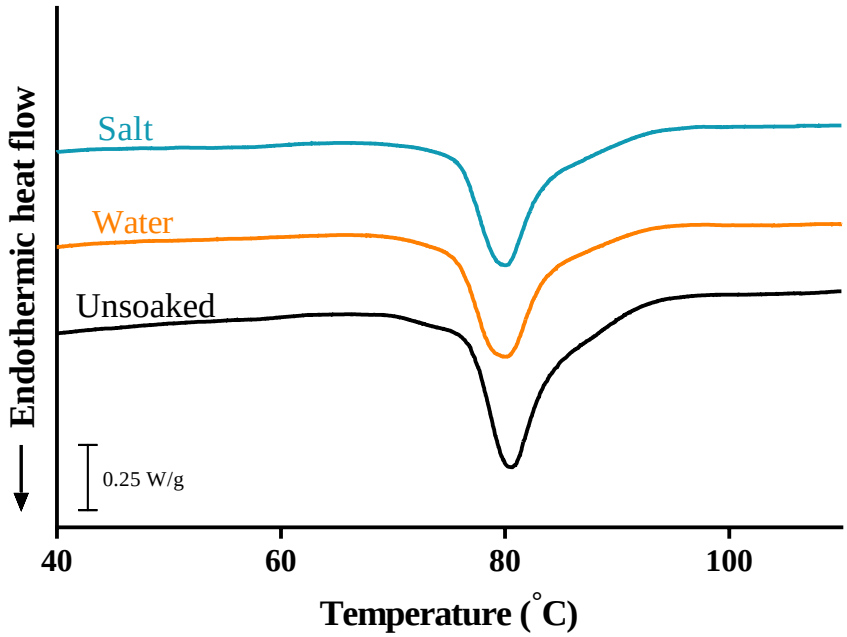

Figure 30 DSC thermograms of unsoaked, DW-soaked, and SS-soaked Bambara groundnut flours.

6.3.4.2 Pectin solubility

To investigate the modification of pectic polysaccharides induced by soaking process, the pectin present in the cell wall materials was extracted into water-soluble pectin (WSP) fraction and chelator-soluble pectin (CSP) fraction. The WSP fraction contains pectin that is loosely bound through hydrogen bonds which can be easily disrupted by heat, whereas the CSP fraction contains pectin that is crosslinked to Ca^{2+} through strong ionic bonds (Njoroge *et al.*, 2014). High amounts of pectin in the CSP fraction have been shown to be associated with the HTC phenomenon in Bambara groundnut (Section 5.3.3).

Figure 31 shows the differences in pectin solubility among unsoaked, DW-soaked and SS-soaked Bambara groundnut samples. The pectin content in WSP fraction increased significantly ($p<0.01$) following soaking process, suggesting that soaking may mediate the conversion of water-insoluble pectin to water-soluble pectin, regardless of soaking medium. Additionally, when compared with the unsoaked and DW-soaked samples, the SS-soaked seeds contained a significantly ($p<0.05$) lower level of pectin in the CSP fraction, implying the possible involvement of Na^+ ions in displacing divalent cations from the carboxyl groups of the pectic polysaccharides (Varriano-Martson and De Omana, 1979; de León, Elías and Bressani, 1992). The CO_3^{2-} may also sequester the divalent cations from the pectate complexes through chelation mechanism, thereby disrupting the crosslinking between divalent cations and pectin (Varriano-Martson and De Omana, 1979). This result is in agreement with the finding of Njoroge *et al.* (2016), who observed a lower CSP fraction in HTC common bean soaked in Na_2CO_3 compared to that soaked in water. A concomitant increase in thermolabile WSP fraction and decrease in insoluble CSP fraction could therefore enhance pectin solubilisation during cooking, eventually leading to a shorter cooking time. Similarly, Uzogara *et al.* (1990) also noted a higher level of pectin solubilisation in cowpea seeds cooked in $NaHCO_3$ and *kanwa* (a mixture of Na_2CO_3 and $NaHCO_3$) solution, as compared to those cooked in distilled water.

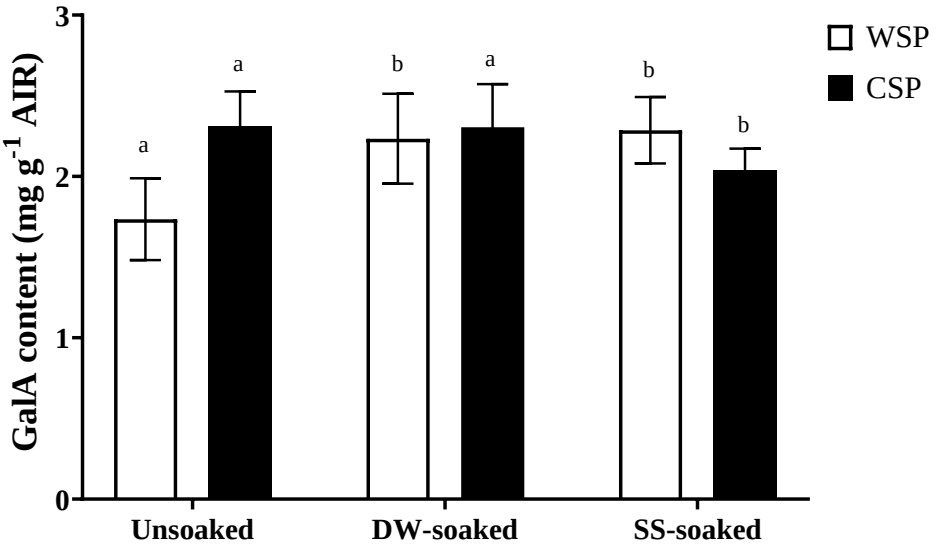

Figure 31 Galacturonic acid content in water-soluble pectin (WSP) and chelator-soluble pectin (CSP) fractions of unsoaked, DW-soaked and SS-soaked Bambara groundnut. Values are means ± SD (n=9). Columns of the same colour with different letters are significantly different ($p<0.05$) with Tukey *post hoc* test.

6.3.5 Effect of soaking medium on cooking behaviour

6.3.5.1 Water uptake and solid loss

Figure 32A shows the progression of water uptake by seeds during cooking. All samples exhibited instantaneous hydration, irrespective of pre-treatment. The disappearance of initial lag phase in water uptake during cooking process could be ascribed to improved seed coat permeability due to increased moisture diffusivity at elevated temperature (Kaptso *et al.*, 2008). The rapid shift in water permeability of seed coat could also be due to the transition of seed coat from glassy to rubbery state, which is enhanced under high temperature (Miano and Augusto, 2018).

Although both pre-soaked samples were imbibed to a similar moisture level ($p>0.05$) before cooking, the DW-soaked seeds did not continue to hydrate at the same rate and to the same extent as did the SS-soaked seeds. The higher water uptake of the SS-soaked seeds during cooking could be attributed to the higher seed pH that may alter the mechanical properties of cell wall polymers, as well as the microstructural properties of the seed coat (Oladele *et al.*, 2018). These effects could be manifested during heat treatment. Meanwhile, the unsoaked sample hydrated substantially faster and reached a higher hydration level than both pre-soaked samples during the first 100 min of cooking, largely due to the high diffusion force driven by high osmotic gradient. For all samples, the osmotic gradient decreased as cooking progressed, leading to a plateau phase after 80 min of cooking.

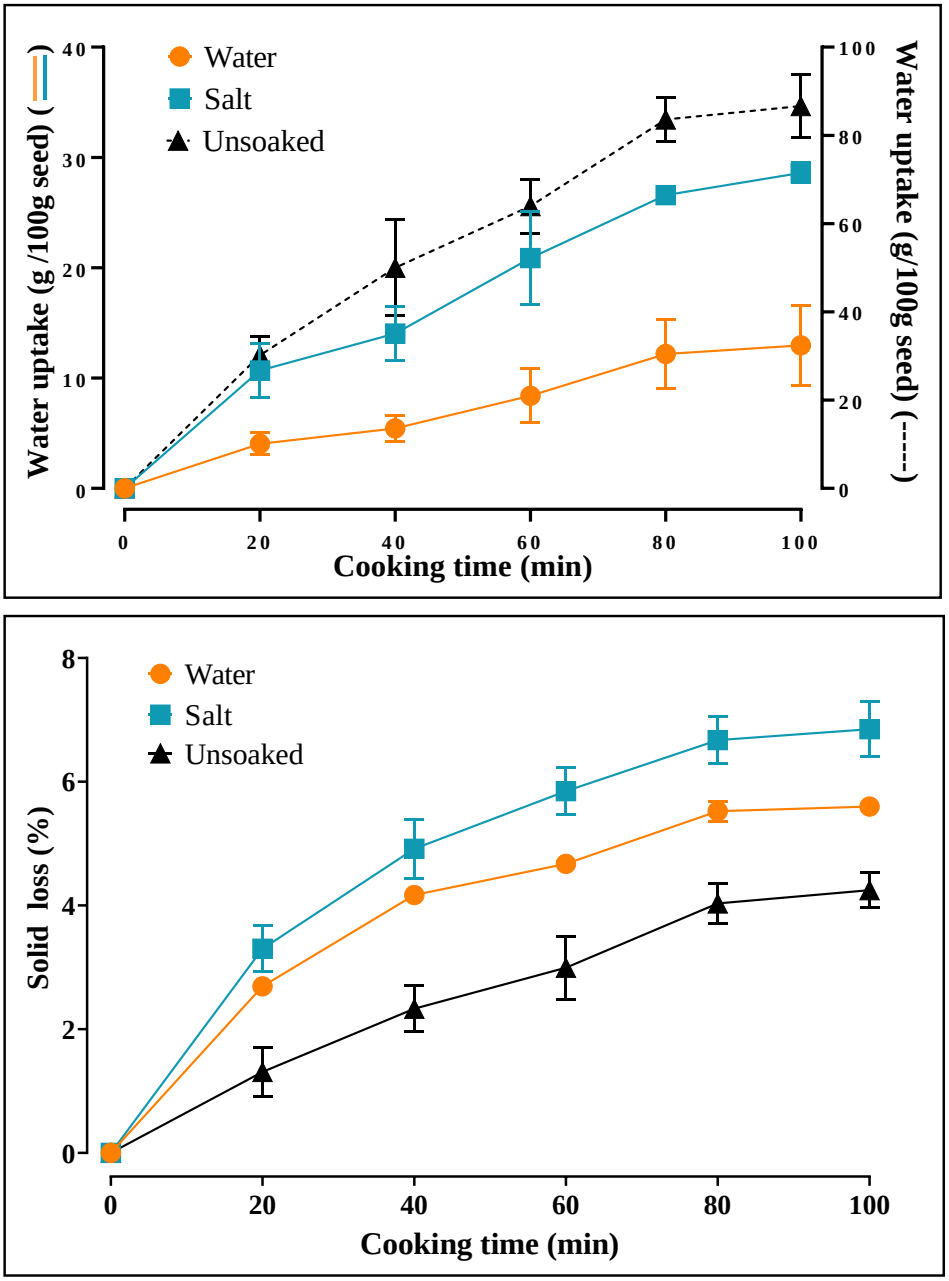

Figure 32 Effect of soaking on water uptake (a) and solid loss (b) of Bambara groundnut seeds during cooking. Markers are the averaged experimental data (n=3), vertical bars represent the standard deviation.

As with water uptake, the curves representing the loss of solids during cooking showed a hyperbolic pattern (Figure 32B). Solid leaching occurred primarily during the first 80 min of cooking and levelled off thereafter. Solid loss was the lowest for unsoaked seeds, probably due to limited rupture of cell walls as a result of restricted pectin degradation (Coffigniez *et al.*, 2019). In contrast, the cell wall materials of SS-soaked seeds exhibited a higher pectin solubility

(Section 6.3.4.2), thus enabling faster dissolution of cell walls during cooking. Enhanced cell wall permeability consequently promoted the loss of solids. Increased protein leaching due to enhanced protein solubilisation might have also contributed to the leaching of soluble solids to cooking medium. Increase in solid leaching has also been reported for cowpea (Uzogara, Morton and Daniel, 1988) and winged bean (Buckle and Sambudi, 1990) cooked in alkaline solution.

High cooking losses have implications for the nutritional quality of Bambara groundnut. Therefore, further study is required to investigate the overall effect of pre-soaking the seeds in salt solution to strike a balance between processing efficiency and nutritional quality of Bambara groundnut.

6.3.5.2 Cooking profile

The cooking profile of Bambara groundnut is illustrated in Figure 33. Overall, DW-soaked seeds took relatively more time to cook than its SS-soaked counterparts, whereas the unsoaked seeds failed to soften within the experimental time. It is worth noting that the % of cooked seeds at 60 min for SS-soaked seeds was averaged at 16%, which was substantially lower than the value predicted earlier in Section 6.3.2 (74.62%). This is likely due to repeated opening and stirring of cooking vessel during cooking process to test the doneness of cooked seeds, hence causing the escape of heat and therefore a slower softening process. On the contrary, the seeds were cooked undisturbed for 60 min in the earlier section.

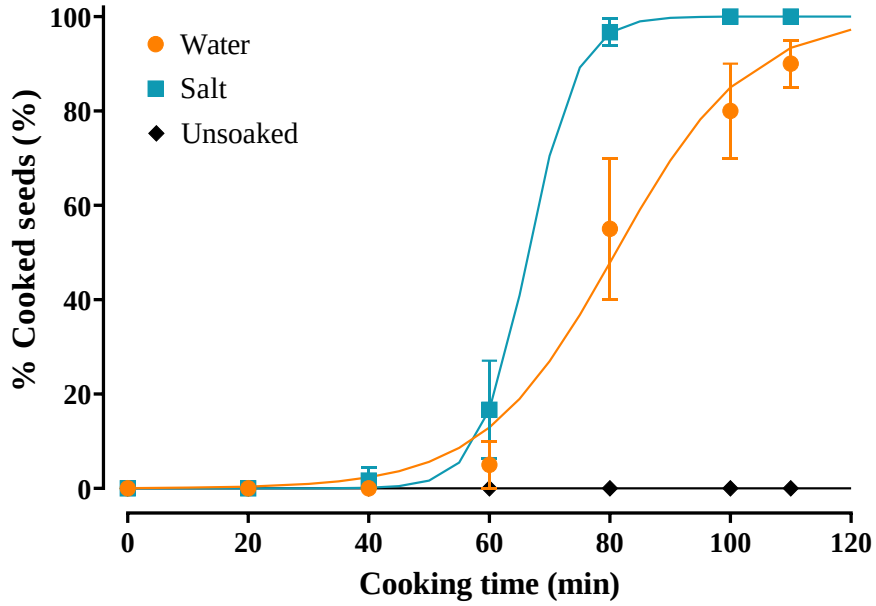

Figure 33 Cooking curves of unsoaked, DW-soaked and SS-soaked Bambara groundnut seeds. Markers are means of experimental values (n=3); vertical bars represent standard deviation; lines denote model-predicted curves (Equation 20).

The cooking curves of both pre-soaked samples exhibited sigmoidal shape and were thus fitted to a sigmoidal model (Equation 20), whereas the unsoaked samples were excluded from the modelling procedures. Fitting the sigmoidal model to the cooking curves showed good fit with

high coefficients of determination (R^2>0.98; Table 16). The estimated values of k_c, the rate constant for cooking, and τ_c, the time required to cook 50% of sample, are presented in Table 16. The lower value of τ_c implies that softening of the cotyledon commenced earlier for SS-soaked seeds than for DW-soaked seeds. In addition, the k_c value for SS-soaked seeds was 2.5-fold higher than that for DW-soaked seeds, indicating a higher rate of cooking compared to the DW-soaked seeds. The hydration level of seeds before cooking was unlikely to account for the differences in the cooking rate, since both samples had reached a similar moisture content ($p>0.05$) prior to cooking.

Table 16 Cooking kinetic parameters of pre-soaked Bambara groundnut estimated by sigmoidal model (Equation 20).

Treatment	k_c (min^{-1})	τ_c (min)	R^2
DW-soaked	0.091 ± 0.017	80.956 ± 2.272	0.980
SS-soaked	0.248 ± 0.011	66.488 ± 0.303	1.000

The reduction in cooking time of SS-soaked seeds can be related to several physiochemical changes brought about by the alkaline salt solution. A higher level of water-soluble pectin, coupled with a lower level of chelator-soluble pectin in SS-soaked seeds (Section 6.3.4.2), suggest a weakened intercellular adhesion, which could aid the dissolution of middle lamella during thermal processing. In addition to affecting the ionically cross-linked pectic polysaccharides, Na_2CO_3 is known to induce de-esterification of pectin by disrupting the ester linkages (Selvendran and O'Neill, 1987), thereby reducing their thermal stability. Furthermore, the SS could shorten the cooking time by modulating the pH of the seeds. The pH of cooking water for unsoaked, DW-soaked and SS-soaked samples at the end of cooking were 5.90, 6.08 and 6.97, respectively, implying a higher pH in the cellular components of SS-soaked seeds. At higher pH, β-eliminative depolymerisation of pectic polysaccharides is enhanced during cooking

(Liu and Bourne, 1995), thus resulting in faster texture degradation. Njoroge *et al.* (2016) reported a greater extent of pectin depolymerisation during cooking of common bean pre-soaked in 0.1 M Na_2CO_3, and partly attributed its shorter cooking time to the higher β-elimination rate. However, it was observed in Section 5.3.3 that the rate of β-elimination might not be a primary factor determining the cookability of Bambara groundnut. Thus, its contribution towards rapid softening of cotyledon in this study requires further investigation. Lastly, alkaline salts have been shown to promote solubilisation of phenolics in Bambara groundnut (Mubaiwa *et al.*, 2019), possibly by breaking the covalent interaction between pectic polysaccharides and phenolic compounds (Yi *et al.*, 2016). The breakdown of these phenolic-pectate complexes, which have been suggested to cause pectin insolubilisation and impede cell separation (Garcia *et al.*, 1998), may also lead to quicker softening of cotyledons.

The gelatinisation of starch, a key event during cooking to render the seeds cooked, requires the presence of water in the starch granules. Hence, the high water uptake rate exhibited by SS-soaked seeds during cooking could also promote starch gelatinisation (Hincks, Mccannel and Stanley, 1987). Additionally, such rapid influx of water during cooking may lead to an increase in turgor pressure of the cells and cause cell expansion, thus accelerating the rate of cell separation (Chen *et al.*, 2021).

6.3.5.3 Colour evaluation

Table 17 presents the colour parameters of cooked Bambara groundnut. The CIELAB L* value of SS-soaked seeds was significantly ($p<0.01$) lower than their unsoaked and DW-soaked counterparts, indicating that soaking in SS had a darkening effect on the cooked seeds. The opposite trend was observed in the redness (a*) values. A simultaneous decrease in lightness and increase in redness implies browning of the seed surface (Arntfield *et al.*, 1997). The darkening effect may be caused by oxidation of bean pigments, or by interaction between the seed chemical components and ions in the solution (Uzogara, Morton and Daniel, 1988). The increase in redness value is also attributable to the liberation of pigmented phenolic compounds during cooking. Leaching of phenolic compounds has been reported to be greater when Bambara groundnut was cooked in alkaline solution (Mubaiwa *et al.*, 2019).

Pre-soaking process resulted in a significant ($p<0.05$) reduction in the yellowness (b*) and colour intensity (chroma, C*) of cooked beans, possibly due to pigment leaching. Hue indicates visual colour perception of the bean, where 0°, 90°,180° and 270° represent red, yellow, green and blue, respectively (McGuire, 1992). All samples had a value close to 90°, indicating that the samples would be perceived as yellow colour. However, a lower hue angle value exhibited by the SS-soaked sample suggests that the seeds possessed red tint to the naked eyes. The ΔE* value quantifies the total colour difference between the pre-soaked and the unsoaked seeds (control). This is an important parameter as consumers are likely to reject a product which has a different colour from the standard colour. According to Hardy and Jideani (2018), a colour difference of $\Delta E^*>1$ indicates that the colour change is noticeable by a viewer, whereas $4<\Delta E^*<8$ is an acceptable range. Hence, both the pre-soaked samples would be deemed acceptable by consumer as their ΔE* values were <5. The result also indicates that pre-soaking Bambara groundnut in appropriate salt solution had no deleterious effect on the colour of the cooked seeds.

Table 17 Hunter colour parameters of cooked Bambara groundnut.

Treatment	CIELAB colour			Chroma, C*	Hue, h (°)	Colour difference, ΔE*
	L*	a*	b*			
Unsoaked	55.16 ± 2.70[a]	3.54 ± 0.95[a]	19.20 ± 2.15[a]	19.54 ± 2.22[a]	79.63 ± 2.25[a]	control
DW-soaked	54.99 ± 4.40[a]	3.22 ± 0.97[a]	16.67 ± 2.19[b]	17.00 ± 2.20[b]	79.02 ± 3.28[a]	2.559
SS-soaked	51.00 ± 2.98[b]	4.63 ± 1.09[b]	16.79 ± 1.29[b]	17.44 ± 1.36[b]	74.63 ± 3.38[b]	4.941

Values are means ± SD (n=20). Values with different superscript letters within a column are significantly different ($p<0.05$).

6.3.5.4 Scanning electron microscopy (SEM)

The microstructure of seed cotyledons was examined using SEM to visualise cotyledon cell separation at different stages of cooking. Two types of fracture modes can be observed from the scanning electron micrographs: intracellular fracture, in which the fracture occurred through the cell walls; and intercellular fracture, in which the fracture occurred along the surface of the cell walls. The intracellular contents of cotyledon cells were exposed in all uncooked samples, with their starch granules were clearly visible (Figures 34Ai; 34Bi; 34Ci). This observation points to the strong intercellular adhesion that resists cell separation. As a consequence, tissue fracture occurs by rupture of cell walls, thus causing exposure of cell contents (Sefa-Dedeh, Stanley and Voisey, 1978). The sizes of starch granules were indistinguishable between samples, with diameter averaging about 30 μm, in agreement with data reported for other Bambara groundnut genotypes (Section 4.3.3.2).

After 20 min cooking, cell breakage (intracellular fracture) remained the dominant mode of fracture for the unsoaked sample (Figure 34Aii). Contrarily, the DW- and SS-soaked seeds exhibited only the exterior cell walls, indicating that cell separation through the middle lamella was favoured during fracturing (Figures 34Bii; 34Cii). This could be the result of pectin solubilisation and degradation during thermal treatment, thus weakening the adhesion between adjacent cells. As a result, stresses applied by mechanical fracture would be imposed on the weaker point, that is, the middle lamella. Previous studies have demonstrated a similar shift in fracturing mode in cooked cowpea (Sefa-Dedeh, Stanley and Voisey, 1978), black common bean (Garcia-Vela, del Valle and Stanley, 1991), and soybean (Koriyama *et al.*, 2017) that had been pre-soaked before cooking. At this stage of cooking, the cotyledon cells for DW- and SS-soaked samples were still densely packed with relatively small and few intercellular spaces, indicating that although the adhesion held by middle lamella had begun to loosen, cell separation had not occurred.

Figures 34Biii and 34Ciii illustrate that cell separation became evident at 40 min of cooking for DW- and SS-soaked seeds. As cooking progressed, cell separation pattern for DW-soaked seeds resembled that of SS-soaked seeds, albeit at a slower rate. Extensive cell separation was observed in the SS-soaked seeds at around 60-80 min cooking, whereas a small extent of adhesion could still be observed for DW-soaked seeds after 100 min cooking (red arrows, Figure 34). The pattern of cell separation is consistent with the cooking curves of the seeds (Section 6.3.5.2).

The cell wall structure of both pre-soaked samples remained intact after 100 min cooking, but pits and folds became apparent on the cell surface of SS-soaked samples after 60 min cooking (blue arrows, Figure 34). Similar observation was made in cooked black common bean that had been pre-soaked in $NaHCO_3$ (Aguilera and Rivera, 1992). Such microstructural changes could be due to enhanced solubilisation and depolymerisation of pectin under alkaline condition, thus weakening the cell wall integrity. Using a light microscope, Chen *et al.* (2021) also visualised cell wall thinning in aged common bean that had been soaked in 0.05M Na_2CO_3 for 10 weeks. The loss of membrane integrity in SS-soaked seeds supports the earlier observation that they displayed extensive solute leaching during cooking (Section 6.3.5.1).

The unsoaked seeds, on the other hand, showed marked differences from the pre-soaked samples. Starch granules remained visible in unsoaked sample up to 60 min of cooking, indicating that cell wall rupture was favoured during tissue fracture. The retention of mechanical strength of the cell wall structure reflects the strong adhesive forces between adjacent cells that are resistant to thermal treatment. Consequently, the cotyledon cells exhibited minimal cell separation and had indistinguishable intercellular spaces during the first 80 min of cooking. The tight arrangement of cotyledon cells may also contribute to the limited solute leaching of unsoaked seeds during cooking. Another distinct structural feature for unsoaked seeds is that upon 60 min of cooking, its intracellular contents appeared to pull away from the cell wall.

creating voids within the cell (yellow arrows, Figure 34), whereas the cell walls (white arrows, Figure 34) retained its rigid form. Such retraction of cytoplasm has been reported in soaked HTC black bean (Varriano-Martson and Jackson, 1981) which the authors attributed to weakened attachment between cell wall and plasma membrane as a result of ageing process. In this study, it is possible that the simultaneous hydration and heat treatment in unsoaked seeds resulted in extensive cell wall swelling, as evidenced by the increasingly distinguishable cell walls at later stages of cooking. It is therefore speculated that the swelling of cell wall occurred more rapidly than the swelling of cytoplasmic contents. Such non-uniformity of swelling therefore caused separation between the cell wall and plasmalemma. During subsequent freeze-drying, the cytoplasmic contents, which no longer remained attached to and supported by the cell wall, contracted more dramatically, resulting in large spaces within the cell.

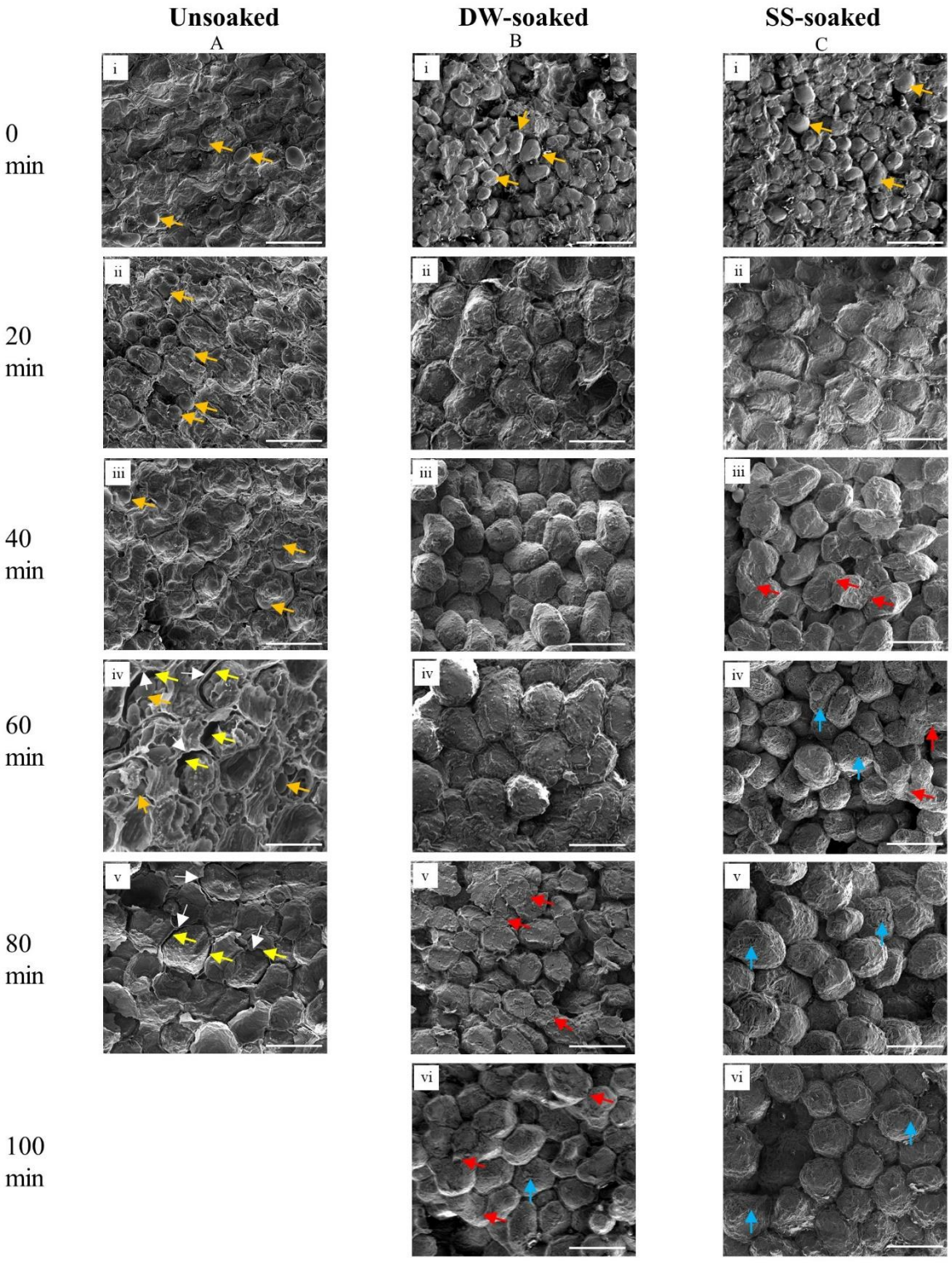

Figure 34 Scanning electron micrographs of unsoaked (A), DW-soaked (B) and SS-soaked (C) Bambara groundnut cotyledons at different cooking times. Orange arrows indicate starch granules, red arrows indicate the remnant of cell adhesion, blue arrows indicate solubilised cell wall, yellow arrows point at the voids between cytoplasm and rigid cell wall (white arrows). Image of unsoaked 100 min (Figure 34 Avi) was not shown due to lack of micrograph. Scale bar = 100 μm.

6.4 Conclusion

Response surface methodology was used to establish the optimal soaking solution to promote hydration capacity and cookability of Bambara groundnut, while minimising its colour change after cooking.

The optimised salt solution (0.25% $NaHCO_3$ and 0.144% Na_2CO_3) had a promotive effect on water uptake of seeds during soaking and cooking, possibly by altering the barrier properties of seeds. This subsequently affected the extent of solute leaching during soaking and cooking, which may have an impact on the nutritional quality of the seeds. Soaking process also induced significant ($p<0.05$) changes in the thermal profile of Bambara groundnut by reducing the T_o and ΔH. Additionally, soaking Bambara groundnut resulted in significant changes in pectin solubility: the water-soluble pectin fraction increased significantly ($p<0.01$) following soaking process regardless of soaking medium, while the chelator-soluble pectin fraction decreased significantly ($p<0.05$) after soaking in salt solution. These changes may have contributed to ease of cell separation during cooking. Consequently, pre-soaking the seeds in alkaline salt solution considerably reduced the cooking time. Distinct differences in the microstructure of seeds at different stages of cooking were observed between the pre-soaked and unsoaked samples. Nevertheless, all samples exhibited gradual weakening of middle lamella and progressive cell separation during the first 100 min of cooking.

In conclusion, this study demonstrates that pre-soaking in salt solution composed of readily available salts ($NaHCO_3$ and Na_2CO_3) is a simple and low-cost treatment in reducing cooking time of Bambara groundnut and would therefore be a practical approach in enhancing the utilisation of the nutritious legume. However, further research is required to assess the effects of salt soaking on the nutritional and sensory quality of the bean.

Chapter 7: Conclusions, study limitations and future studies

7.1 Conclusions

The aim of this study was to gain a deeper insight into the hard-to-cook phenomenon of Bambara groundnut. The first experiment of this study was undertaken to explore the variability in quality attributes among 12 Bambara groundnut genotypes (Chapter 4). Physical characteristics, hydration behaviour, microstructure of seed morphology and cooking quality were investigated. The genotypes exhibited different degrees of variation in terms of the geometric dimensions and gravimetric properties. Notably, the 100-seed weight showed an almost two-fold variation among the 12 genotypes (50.24-92.30 g). The geometric mean diameter, on the other hand, fell within a relatively narrow range of 9.40-11.35 mm. Sphericity of the seed, which is an indicator of seed shape, were generally similar across all genotypes, ranging between 0.82 and 0.92. Seed coat percentage and thickness were negatively correlated ($p<0.01$) with seed coat lightness (CIELAB L*), indicating that light-coloured genotypes tended to have thinner testae.

Although microscopic cracks were observed on the seed coat of all Bambara groundnut genotypes, the seed coats remained impermeable to water at low moisture levels (<10% dwb) and impeded the initial water uptake of the seed. Hilum was found to be the primary path of initial water entry into the seed. Due to its relatively small surface area as compared to the overall seed surface area, and to its narrow groove, water uptake was slow during the early stages of hydration, causing a lag phase before the start of water imbibition. As the moisture content of the seed increased (>30% dwb), the seed coats became permeable, causing a faster water imbibition rate (i.e., an exponential phase). When the seed achieved the saturation moisture content, water uptake ceased, resulting in a plateau phase. This water uptake behaviour exhibited by all Bambara groundnut genotypes led to a sigmoid-shaped hydration curve, which can be described using a

sigmoidal model (Kaptso *et al.*, 2008). When the 12 genotypes were grouped based on their testa colour, the results of the water absorption kinetics revealed that cream-seeded genotypes experienced a shorter lag (τ = 9.6-18.7 h) and imbibed faster (0.158-0.272 h^{-1}) than other genotypes, achieving equilibrium moisture content (99.7-109.6% dwb) within 48 h of soaking. On the contrary, the red-coloured genotypes had a considerable lag time (τ = 20.2-24.6 h) and an intermediate water uptake rate (0.129-0.131 h^{-1}) but attained the highest equilibrium moisture content (121.9-135.2% dwb) among all genotypes. The black-coloured genotypes were mostly slow hydrating (0.111-0.146 h^{-1}) and had a longer lag phase (τ = 19.6-25.7 h), with an appreciable level of equilibrium moisture content (106.2-121.3% dwb). The brown-seeded genotype, however, was outstanding for exhibiting the lowest hydration rate (0.095 h^{-1}) and equilibrium moisture content (94.5% dwb) while having an extended lag period (τ =24.7 h).

The microstructure of the seed may provide some explanations to the genotypic variation in hydration behaviour. For instance, when viewed under the SEM, the genotypes with rapid imbibition rates appeared to have bigger tracheid bars under the hilar groove, which may allow a higher rate of water influx into the seeds during the initial soaking stages. Additionally, the cotyledon of these genotypes was more loosely packed, containing higher numbers and bigger sizes of intercellular spaces, which may facilitate capillary flow during the soaking process. Since the information on the microstructure of Bambara groundnut is scarce in the literature, the micrographs obtained in this study may be useful for future studies.

The cooking times (CTs) varied between and within genotypes. The fully hydrated seeds cooked faster (38-120 mins) and more uniformly compared to the partially hydrated seeds (70-208 mins). A positive correlation ($r=0,582$, $p<0.05$) between CT and leaching losses during soaking was obtained, supporting the cell membrane degradation hypothesis (Hentges, Weaver and Nielsen, 1991). The lack of correlations between the cookability of fully hydrated seeds and their physical and hydration kinetics parameters led to the suggestion that chemical properties

may be responsible for the differences in cooking time among the genotypes. The absence of relationship also indicates that the physical traits are not useful in predicting the cookability of Bambara groundnut. Nevertheless, the detailed investigation into the physical, microstructural, and technological properties across the genetically diverse Bambara groundnut genotypes may provide useful data for future breeding programmes.

Following the initial screening on the cooking quality, four genotypes were selected for the second experiment, which sought to examine the compositional differences between easy-to-cook (ETC) and hard-to-cook (HTC) genotypes (Chapter 5). The ETC genotypes, namely C_KARO and R_SONG, had an average cooking time between 38 and 43 min; while the HTC genotypes, namely B_IPBB and N_ANAM, had an average cooking time between 80 and 120 min. In contrast to the protein-starch hypothesis which postulates that the decline in protein solubility of pulses is a possible cause of the HTC phenomenon (Liu, McWatters and Phillips, 1992), the protein solubility did not show significant variation between genotypes (33.74-38.09%; $p>0.05$). Additionally, the ETC and HTC genotypes had similar ($p>0.05$) composition of protein secondary structure, implying that this parameter did not influence the CT of Bambara groundnut. The DSC thermograms of the Bambara groundnut flours showed a single endotherm (T_{peak} = 80.1-83.6°C), which may be the result of both starch gelatinisation and protein denaturation occurring concurrently within the range of thermal transition. Hence, it was also not possible to draw a firm conclusion on the thermal stability of protein. Nonetheless, a significantly ($p<0.05$) higher T_{peak} and $T_{conclusion}$ was observed for the HTC genotypes, suggesting that the biomolecules (starch and protein) in HTC genotypes were more thermally stable. The significantly ($p<0.05$) higher ΔH values among the HTC genotypes indicate a higher energy requirement for the biomolecules to undergo thermal transition.

The pectic polysaccharides present in the cell wall materials (extracted as alcohol-insoluble residue, AIR) was also studied. The total pectin content (56.22-63.64 mg GalA g^{-1} AIR)

and the degree of methyl-esterification (DoM) of pectin (37.01-56.44%) could not explain differences in cooking time between genotypes. The solubility of pectin, however, was associated with the cooking time. The ETC genotypes contained a higher ($p<0.01$) level of water-soluble pectin and a lower ($p<0.05$) level of chelator-soluble pectin, suggesting a weaker intercellular adhesion. This would potentially enhance the cell separation during cooking, thus resulting in a shorter cooking time. On the one hand, the elevated level of chelator-soluble pectin in the AIR of HTC genotypes concurs with the pectin-cation-phytate hypothesis, which postulates a concomitant increase in chelator-soluble pectin and decline in DoM of pectin during seed hardening process (Reyes-Moreno, Paredes-López and Gonzalez, 1993). On the other hand, in contrast to the hypothesis, the DoM of pectin among the genotypes did not correspond to the ease of cooking. These results suggest that the pectin-cation-phytate model may not explain the hardening phenomenon in Bambara groundnut. Additionally, the molecular structure of the AIR was analysed using ATR-FTIR. This was the first study where ATR-FTIR was applied to the cell wall materials of Bambara groundnut. Although it did not yield conclusive results in terms of explaining the differences in cooking time, the result can be referenced for future studies.

Chapter 6 demonstrates the feasibility of using commonly available salts in improving the processing quality of Bambara groundnut. Genotype C_NAV4 was used in this study. The first part of the study was carried out to obtain an optimised soaking solution to maximise the hydration capacity (HC) and cookability of the seeds (%C) while minimising the colour changes (ΔE) of the cooked seeds. Three salts were used for the experiments: NaCl, $NaHCO_3$ and Na_2CO_3. Results revealed that: (1) HC (55.93-76.46%) increased with the concentrations of Na_2CO_3 but decreased as the concentrations of NaCl increased; (2) %C (56-82%) increased with the concentrations of $NaHCO_3$ and Na_2CO_3 but decreased as the concentrations of NaCl increased; and (3) ΔE of cooked seeds (3.523-9.746) increased with increasing concentrations of all three salts. Using the response surface methodology, the most desirable (desirability = 0.732)

salt solution was obtained (0.25% $NaHCO_3$ + 0.14% Na_2CO_3) and was used in the subsequent experiment.

The second part of the experiment was conducted to evaluate the effect of the optimised salt solution on the processing quality (hydration behaviour and cooking characteristics) of Bambara groundnut. Distilled water was used as control. The use of salt solution during soaking process shortened the lag phase (τ = 8.1 h) and increased the hydration rate (k = 0.211 h^{-1}) of the seeds as compared to distilled water (τ = 10.9 h; k = 0.181 h^{-1}) but did not affect the equilibrium moisture content (94.52-94.55% dwb). A higher level of solute leaching into the salt solution was also observed throughout the soaking and cooking processes. With respect to the thermal properties, the seeds pre-soaked in distilled water and those pre-soaked in salt solution showed a similar ($p>0.05$) thermal profile. This result implies that the use of the salt mixture did not modify the thermal behaviour of the seeds. The level of chelator-soluble pectin, however, was greatly reduced ($p<0.05$) after soaking in salt solution. The changes in pectin solubility may partially explain the greater cooking rate (increased by 2.5 times) exhibited by the salt solution-soaked seeds, as compared to the distilled water-soaked seeds. The cooking profile of the seeds was supported by the microscopic result, which demonstrated that the addition of salts to the soaking solution facilitated cell separation during cooking, thereby resulting in a shorter cooking time. Despite that the addition of alkaline salt to the soaking solution resulted in darkening of the cooked seeds, the overall colour change (ΔE^* = 4.941) was within the acceptable range.

7.2 Limitations of current work

The current work has laid the groundwork for the investigation of hard-to-cook phenomenon in Bambara groundnut. There are, however, limitations in the present work that need to be acknowledged:

1. The seed cooking time has been determined using subjective method (finger pressing and white core method), which is subject to high variability between laboratory personnel (e.g., pressing force exerted by individuals, definition of "soft"). The use of objective methods, such as Mattson bean cooker or texture analyser, could increase accuracy and improve the reproducibility of the results.
2. The current study only investigated genotypic variation in cooking quality as it was not feasible to monitor the changes throughout prolonged storage period due to time constraints. It may be necessary to consider the effects of genotype, and storage time and conditions to elucidate the mechanism of HTC phenomenon in Bambara groundnut. Ideally, growing environment should also be taken into consideration.
3. Time constraints and several national lockdowns due to Covid-19 pandemic have impeded the cultivation of Bambara groundnut, causing limited types of samples used in the current study. Screening a larger number of genotypes could produce more reliable data in pinpointing the factors associated with genetic variability of cooking time.

7.3 Recommendations for future studies

Selection of fast-cooking genotypes is a key component for breeding programme. This could be accomplished by consolidating existing understanding on the mechanisms associated with HTC phenomenon. Developing preventive measures of HTC phenomenon and effective approaches to overcome the hardening phenomenon are also crucial to improve utilisation of the crop.

Suggestions for future research:

1. To study other factors causing susceptibility to HTC development. For instance, genotypes (gene associated with pectin solubility), growing location (Ca, Mg and P contents in soil), growing season (drought vs rainy season), and maturity of seed.

2. To monitor the development of HTC phenomenon throughout prolonged storage period. For instance, it would be worthwhile to monitor the changes in tissue pH and protein solubility, or changes in pectin solubility and degree of methyl-esterification of Bambara groundnut during adverse storage.
3. To study the contribution of seed coat to the cooking time and the texture of cooked seeds. Given the relatively thick seed coat of Bambara groundnut, it would also be of interest to study the effect of milling, which involves seed coat removal and cotyledon splitting, on the ease of cooking.
4. To monitor the extent of starch gelatinisation and protein denaturation during cooking. Cell separation during cooking has been visualised in Section 6.3.5.4. Apart from cell separation, starch gelatinisation and protein denaturation are important events during cooking to render the seed cooked. For instance, the degree of starch gelatinisation can be assessed by studying the residual birefringence under polarised light, while the extent of protein denaturation can be estimated by determining the protein solubility.
5. To assess the leaching of nutrients and ANFs during soaking and cooking, particularly when using salt solution. It is also crucial to investigate the bioavailability of nutrients in cooked seeds.
6. To devise methods to utilise the HTC seeds – for instance, extrusion.
7. To devise low-cost storage method to delay the development of HTC.

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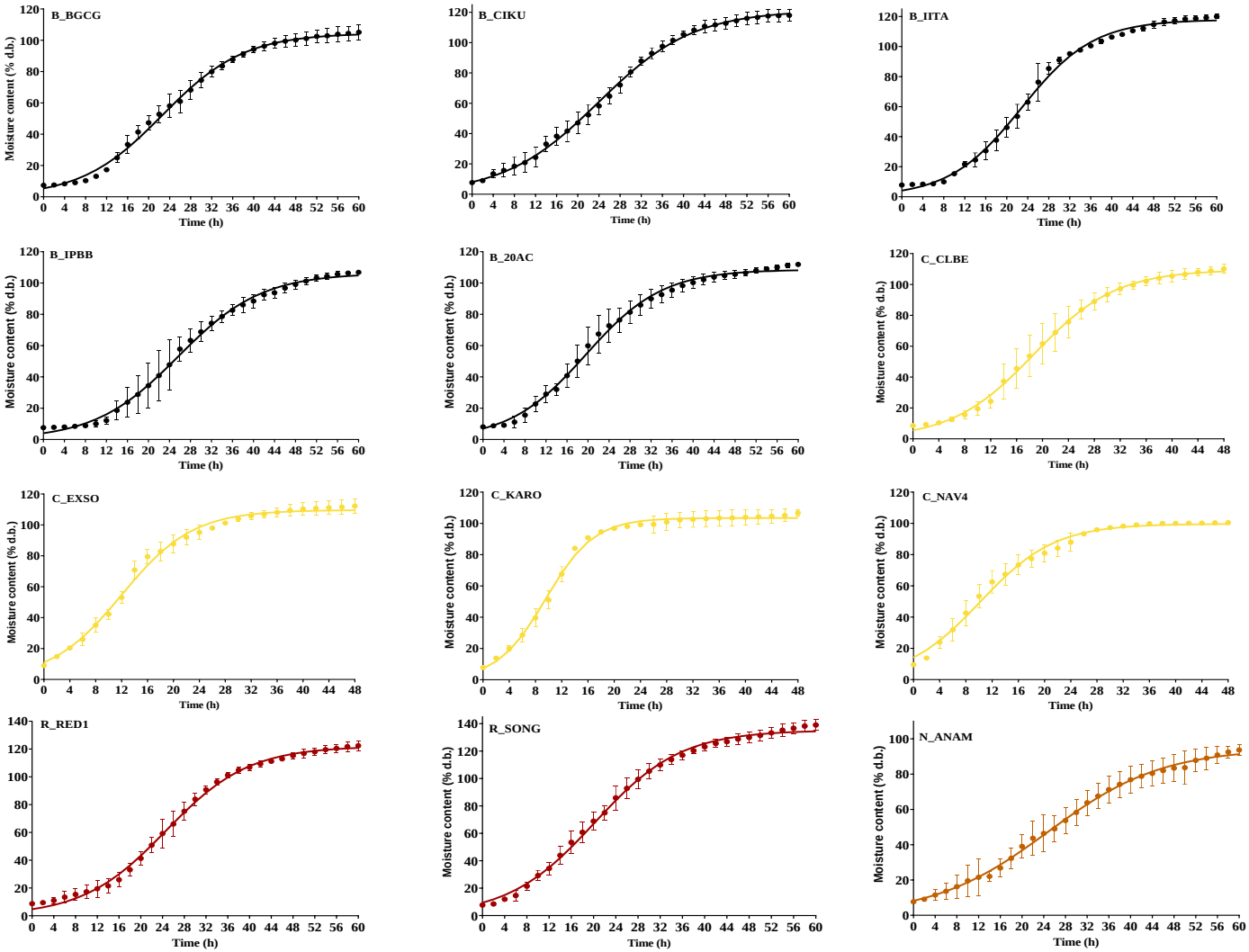
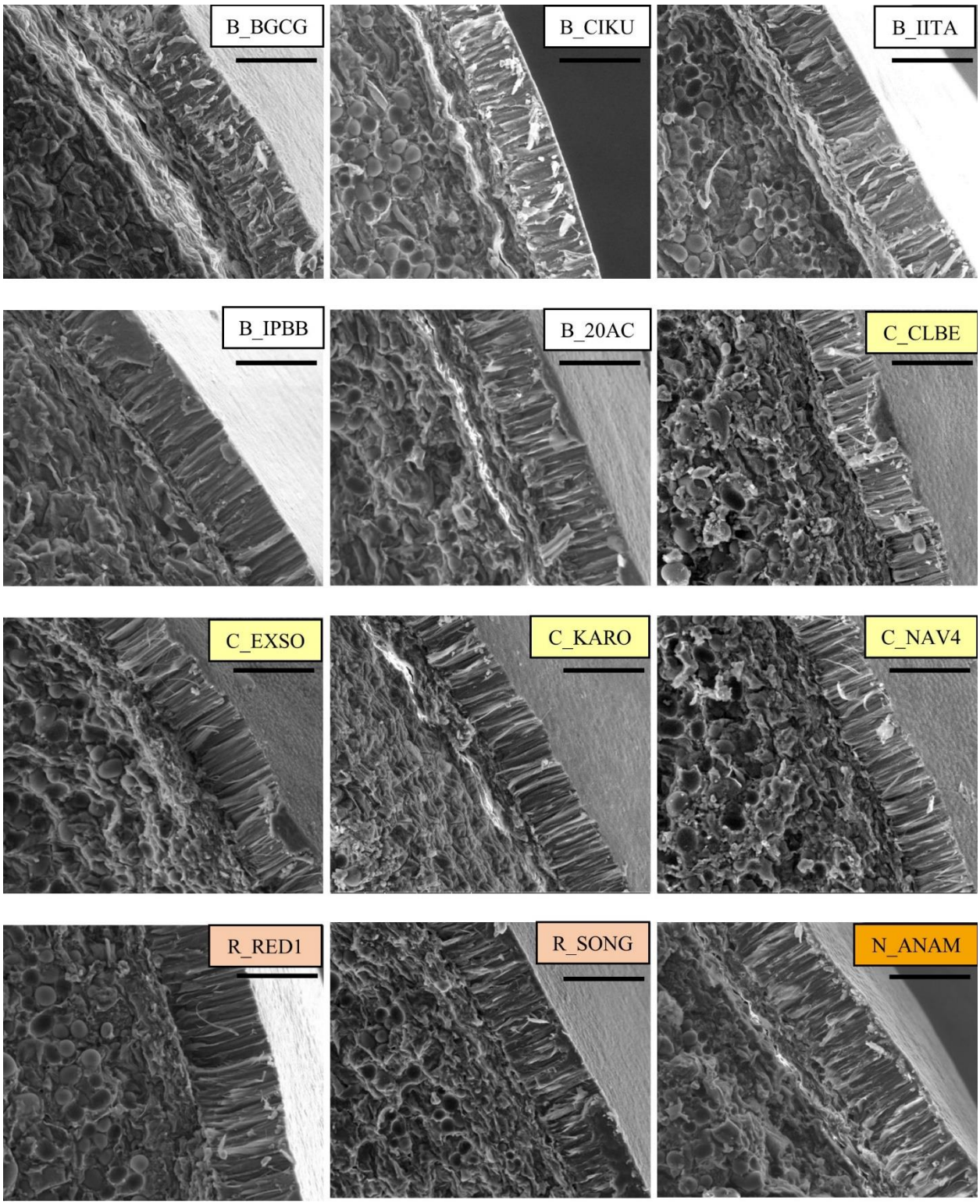
APPENDICES

Appendix 1: Individual plots of water absorption curves of 12 Bambara groundnut genotypes.



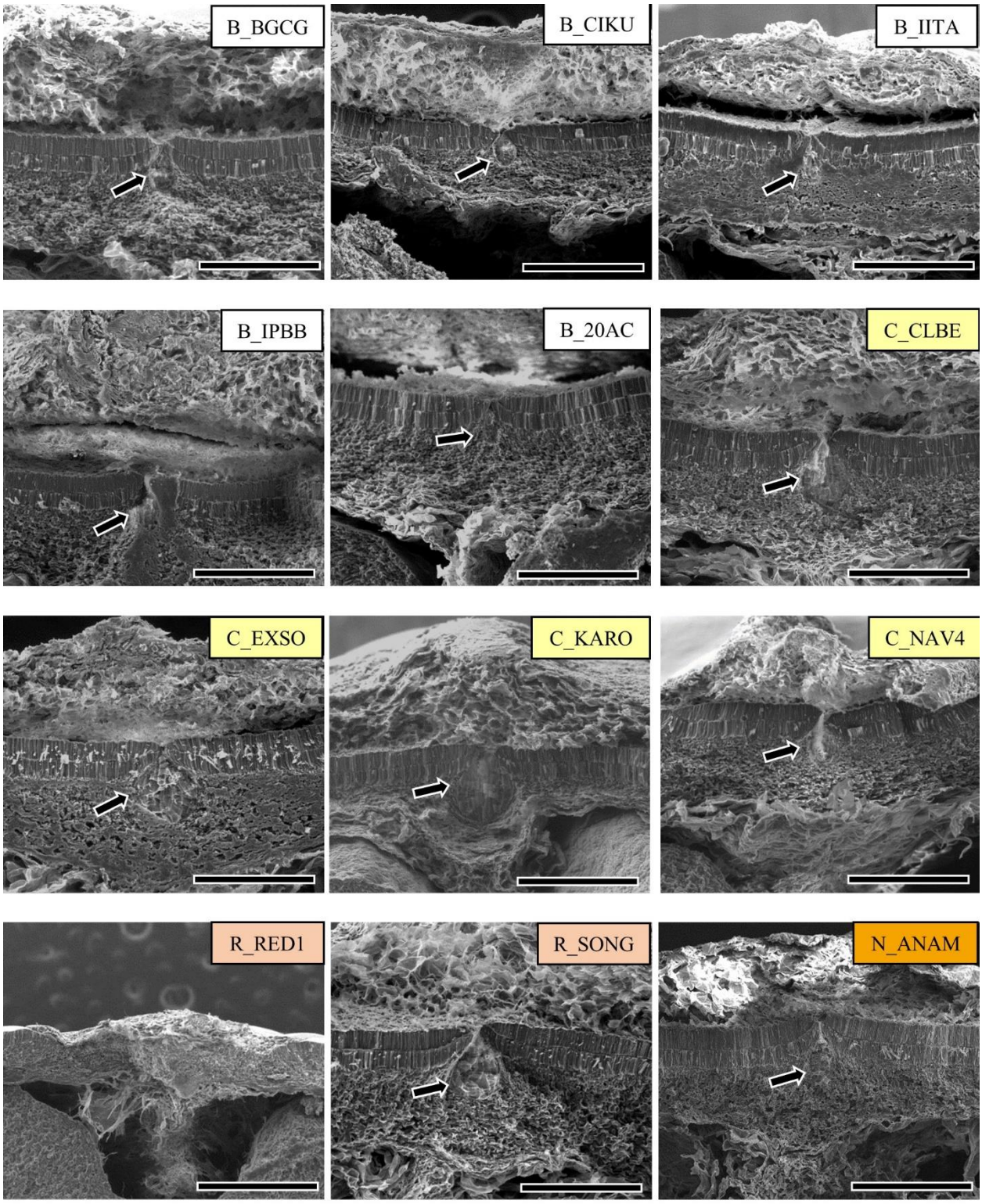
Markers are the averaged experimental data; vertical bars indicate standard deviation; solid lines are curves predicted by Equation 13. Black, yellow, red and brown graphs represent black-, cream-, red-, and brown-coloured seed coats,

Appendix 2a: Cross-sections of Bambara groundnut seed coat and cotyledon of 12 genotypes.



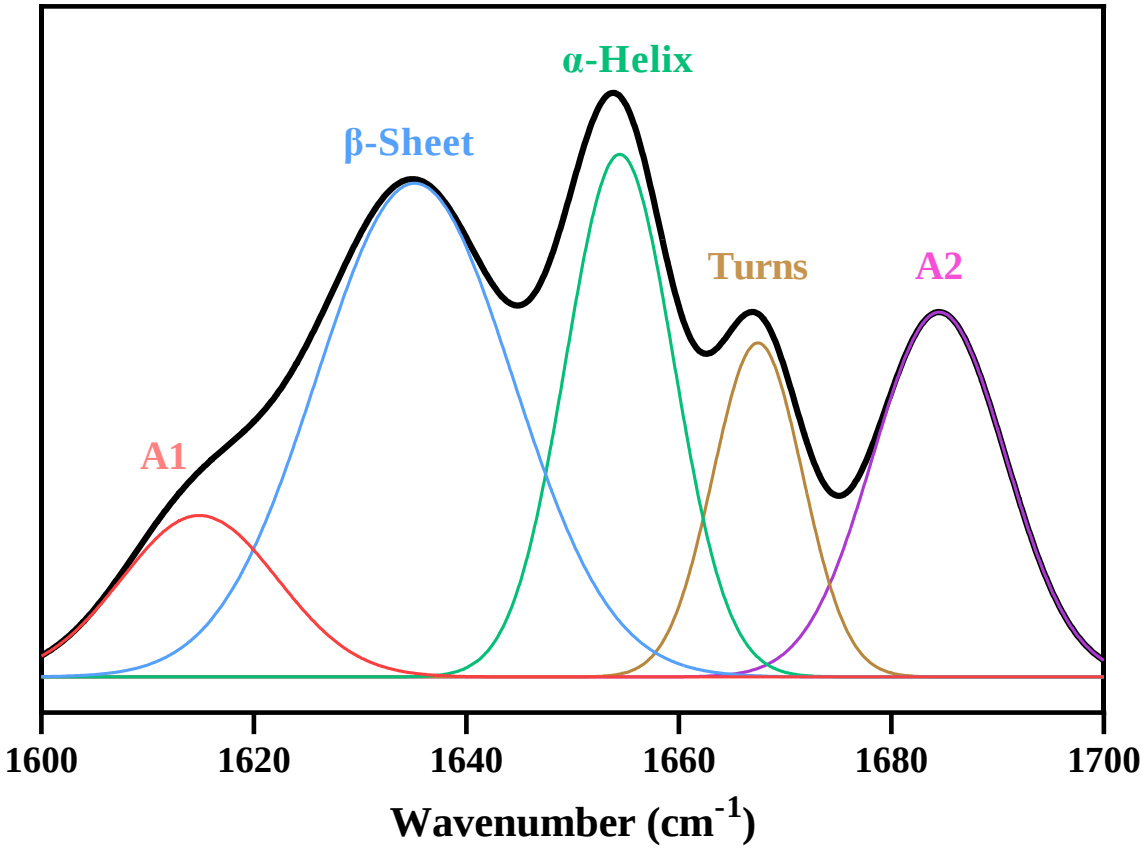
Genotype names with white, yellow, red and brown labels represent black, cream, red and brown genotypes, respectively. Bar = 100 μm.

Appendix 2b: Cross-sections through hilar region of 12 Bambara groundnut genotypes.

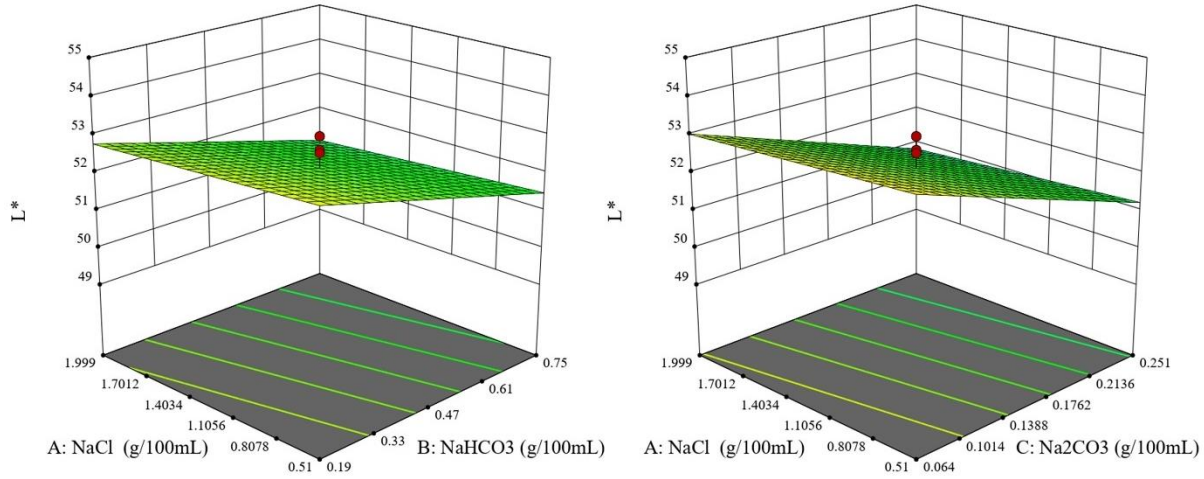


Arrows indicate the tracheid bar. Genotype names with white, yellow, red and brown labels represent black, cream, red and brown genotypes, respectively. Bar = 400 μm.

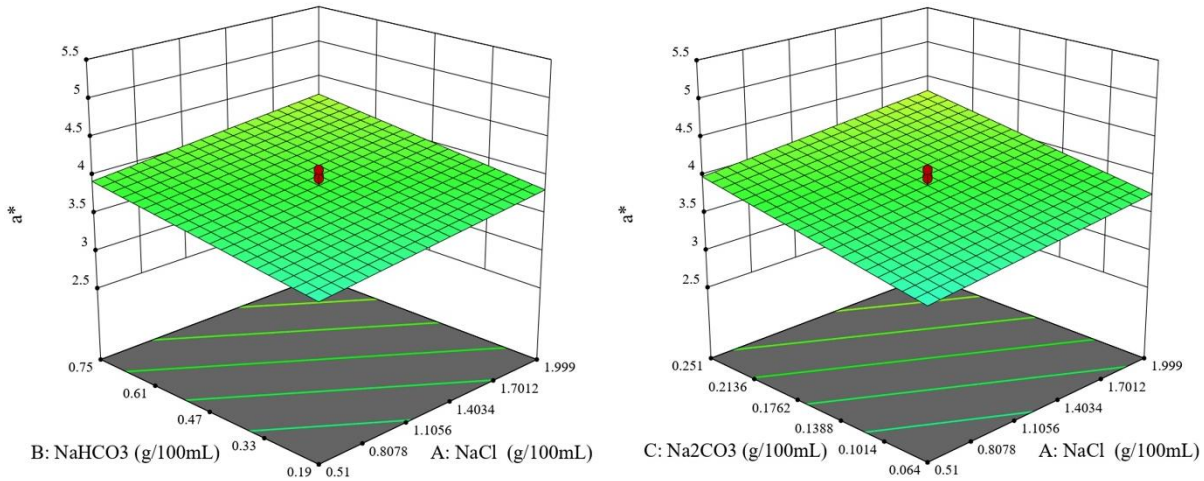
Appendix 3: Deconvoluted and curve fitted FTIR spectrum of N_ANAM flour in the amide I region. Gaussian lines represent the protein secondary structures.



Appendix 4: Response surface plots of CIELAB colour parameters as a function of NaCl and $NaHCO_3$ and Na_2CO_3 concentrations.



Appendix 4a Response surface plots of CIELAB L* as a function of (A) NaCl and $NaHCO_3$ concentrations and (B) NaCl and Na_2CO_3 concentrations. Equation for the model was: $L^* = 52.13 - 0.2023X_1 - 0.8487X_2 - 1.11X_3$, where X_1, X_2 and X_3 represent the concentrations of NaCl, $NaHCO_3$ and Na_2CO_3, respectively. R^2=0.6987; lack-of-fit p>0.05. The effect of concentrations of $NaHCO_3$ and Na_2CO_3 on L* were both significant at p<0.0001.



Appendix 4b Response surface plots of CIELAB a* as a function of (A) NaCl and $NaHCO_3$ concentrations and (B) NaCl and Na_2CO_3 concentrations. Equation for the model was: $a^* = 3.88 + 0.1594X_1 + 0.2150X_2 + 0.2743X_3$, where X_1, X_2 and X_3 represent the concentrations of NaCl, $NaHCO_3$ and Na_2CO_3, respectively. R^2=0.4513; lack-of-fit p>0.05. The effect of concentrations of $NaHCO_3$ and Na_2CO_3 on a* were significant at p<0.05 and p<0.01, respectively.