Nutritional and functional properties of soaked and micronized Bambara groundnut seeds and their flours

By

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DECLARATION

I declare that the thesis which I hereby submit for the degree PhD (Food Science) at the University of Pretoria is my own work and has not previously been submitted by me for a degree at another university or institution of higher education.

Opeoluwa Mayowa Ogundele

SIGNATURE:……

DATE:……

ABSTRACT

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Bambara groundnut (*Vigna subterranean L.*) is considered a good source of protein in some parts of sub Saharan African countries. Long cooking time of about three hours contributed to its limited consumption and utilisation. Micronisation is an infrared heating process. It can reduce the cooking time of pre-moisture conditioned legume small seeds size such as cowpea and lentil, but mostly tempering of seeds has been used as the pre-conditioning techniques. The resulting flour from the pre-conditioned micronised cowpea can reduce pasting viscosity and has potentials in food systems as an instant product. This study aimed at (1) determining the effects of micronisation of pre-soaked whole and dehulled bambara groundnut seeds on their cooking characteristics, (2) determining the effects of micronisation and dehulling treatment of pre-soaked bambara groundnut on physicochemical, microstructure and functional properties of the resulting flours and (3) determining the effects of micronisation of pre-soaked whole and dehulled bambara groundnut seeds on the viscosity, nutritional and health benefits of the cooked samples in order to produce a quick cooked bambara groundnut with functional, nutritional and health benefits.

Micronisation (130 ºC) at a different time (0, 5, 10 and 15 min) was used to optimise the process for pre-soaked (53% moisture) bambara groundnuts. Micronisation (130 ºC) reduced cooking time of pre-soaked (53% moisture) bambara groundnut (whole and dehulled) following cooking. Micronisation reduced the 162 min cooking of raw bambara groundnut to 109, 83, 75 and 62 min when micronized for 0, 5, 10 and 15 min. Micronisation (53% moisture, 130 ºC) caused

molecular changes such as solubilisation of pectin which was responsible for the disruption of the middle lamella and separation of parenchyma cell observed by light microscopy and scanning electron microscopy (SEM). It also caused disruption in the structure of starch granule, protein matrix in the cotyledon. These changes in seeds structure and molecular properties of starch, protein and pectin, facilitate water hydration rate and cell separation during cooking, leading to the shorter cooking time of the bambara groundnuts.

Micronisation of pre-soaked (53% moisture) bambara groundnuts caused molecular changes such as partial starch gelatinisation and reduced protein solubility in the resulting flours. The changes in the starch and proteins modified the resulting flours functional properties such asincrease swelling of the resulting flours, while reducing the water solubility. The pasting viscosities of resulting flours of pre-soaked bambara groundnut reduced following micronisation due to the denatured protein matrix preventing embedded starch hydration, dispersibility and molecular entanglement during pasting.This was evident by light and confocal laser scanning microscopy (CLSM) that showed the aggregates of denatured protein matrix surrounding embedded pre-gelatinised starch granules increase with micronisation in the resulting flours and cooked soft porridge of bambara groundnut.

Micronisation has an effect on the apparent viscosity, nutritional, bioactive compound such as phenolics and hence the antioxidant properties of cooked soft porridge of bambara groundnut. All cooked soft porridge of bambara groundnut exhibited a shear thinning behavior and micronised bambara groundnut had lower viscosity increased in the starch and protein digestibility of cooked soft porridge of bambara groundnut. It also increased the phenolic content and antioxidant properties of cooked soft porridge of whole bambara groundnut, but these were reduced in cooked soft porridge of dehulled bambara groundnut due to seed coat absence.

Thus, micronisation of pre-soaked bambara groundnut (whole and dehulled) would contribute towards increased utilisation of bambara groundnut as well as improving house hold nutrition and health promoting properties.Micronisation of bambara groundnut has potential to produce a quick paste with low viscosity which depend on the pre-soaking and micronisation time of the bambara groundnut. Flours from micronised bambara groundnut can therefore be used as instant flour ingredient in food products.

DEDICATION

This thesis is dedicated to the Lord Almighty, the source of my strength throughout this journey.

To the memory of my late supervisor, Prof. Amanda Minnaar.

May her soul rest in peace.

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All my friends, for your moral support throughout the study period. I say thank you. Your support and encouragement meant a lot and may God bless you all.

Finally, my Heavenly Father, God Almighty, for giving me Immeasurable grace to finish this race strong. To you be all the glory.

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1 INTRODUCTION

Bambara groundnut is an indigenous African legume. Its botanical name is *Voandzeia subterranean (L.) thousars* synonymous to *Vigna subterranean* and belongs to the plant family of *Fabaceae* and subs family of *Faboidea* (Borget, 1992). The crop is widely cultivated in sub-saharan Africa and in many parts of South America, Asia and Oceania (Baudoin & Mergeai, 2001). Africa remains the major producer of bambara groundnut with an estimated of about 280,000 tons per year (FAO, 2014). Bambara groundnut seed is known as *Njugo* in South Africa and in Nigeria as *Okpaotuanya*in Ibo language, *Epiroro*in Yoruba language and *Guijiya* in Hausa language (Barimalaa et al., 2005). In Africa, bambara groundnut is ranked as the third most important legume after groundnut *(Arachis hypogea)* and cowpea *(Vigna unguiculata)* (Lacroix et al., 2003). However, it is still underutilised as compared to legume such as cowpea. Bambara groundnut has several agronomic advantages as it is drought tolerant and it possesses the ability to grow in soils considered infertile for the cultivation of other preferred legume species such as groundnuts *(Arachis hypogea)* and common beans (Anchirinah et al., 2001).

Bambara groundnut is rich in dietary protein of about 14–24 g/100g (Lacroix et al., 2003) and also noted as an important source of phytochemicals such as phenolics which have antioxidant properties (Diedericks & Jideani, 2014). Bambara groundnut is commonly eaten fresh, semi-ripe and as fully matured seeds by cooking or roasting. However, the utilisation of bambara groundnut is limited due to its long cooking time (Adebowale et al., 2011; Ojimelukwe, 1999).Cooking bambara groundnut seeds can take as much as three hours or more (Annan et al., 2002). The long cooking time of the legume seeds takes up a lot of fuel energy (Aguilera & Stanley, 1985). Also, the long preparation time of legume seeds as meal remains a challenge to consumers in rural areas who cannot afford the energy costs, and for urban consumers whose busy life style makes convenience an important factor in food choices (Kayitesi et al., 2012).

Micronisation is a short-time high-temperature hydrothermal processing method otherwise referred to as infrared heatingdirected at reducing the cooking time of legumes. It requires simultaneous heat and mass transfer to achieve complex chemical changes taking place during processing (Cenkowski et al., 2003). The mid infrared energy of about 1.8–3.4 µm wave length is passed intothe moisture conditioned grains, the energy is absorbedby the water molecules due

to its dielectric properties and vibrate at a frequency of 8.8×10^7–1.7×10^8 MHz (Fasina et al., 2001). These increased molecular vibrations and intermolecular friction causes rapid internal heating and a rise in water vapour pressure inside the grains (Fasina et al., 1999).In most research, micronisation of whole pre-conditioned legume seeds were done at 30% moisture levels and little information at about 40%. At about 40% of whole preconditioned legume seeds, micronisation have caused different changes in the physical, physicochemical and chemical properties of micronised pre-conditioned lentils (Scanlon et al., 2005) and cowpea (Mwangwela et al., 2006). As results, micronisation was found to improveseeds hydration during soaking and reducelong cooking time of some legumes such as lentils (Cenkowski & Sosulski, 1997; Arntfield et al., 2001), cowpeas (Phadi, 2004; Mwangwela et al., 2006) and split peas (Cenkowski&Sosulski, 1998). However, bambara groundnut seedare compared to other smallerlegume seeds such as cowpea and lentil suggest that micronisation effects may be different.

According to Brough et al. (1993), novel forms of bambara groundnut utilisation might increase its production. The flour of bambara groundnut has the potential of being processed as akara (fried bean cake) (Alobo, 1999), Okpa (steamed stiffed porridge) (Barimalaa et al., 2005), blend in production of bread (Alozie et al., 2009), cookies (Kiin-Kabari & Giami, 2015) and soft porridge for infant (Nnam, 2001a). The suitability of bambara groundnut flour in development of different products is attributed to its functionality in terms of hydration, foaming gelation, emulsification and pasting properties (Eltayeb et al., 2011; Aremu et al., 2007; Sirivongpaisal, 2008). This functionality is a related function of the major macromolecules such as starch and protein. In addition to micronisation reducing cooking time of moisture conditioned whole legume seeds, its effect on biomolecules such as starch and protein has shown to affect functionality of its resulting flours (Mwangwela et al., 2007a; Mwangwela et al., 2007b; Cenkowski & Sosulski, 1998) and could affect the nutritional (Vilakati et al., 2015; Khattab et al., 2009; Khattab& Arnfield, 2009; Wiriyaumpaiwong et al., 2004) and bioactive properties (Kayitesi, 2013) as well. However, little is still known about the functional, nutritional and bioactive properties of resulting flours from micronised pre-soakedlegume seeds. This research project deals with micronisation of pre-soaked whole and dehulled bambara groundnut seeds. The cooking properties of the micronised bambara groundnut seeds are studied. The functional, nutritional and health benefits of the resulting flours from the micronised seeds is also studied.

2 LITERATURE REVIEW

This chapter presents a review on bambara groundnut seeds and flour as a protein source, itsstructure, cooking characteristics, nutritional and health benefits and relative effects of micronisation will be discussed. It is noted that there is limited literature on bambara groundnut. However, cowpea as one of the example of legumes was referred to as alternative specific legume seed in some parts of this review because both are hardy drought tolerant legume seeds belonging to same *Vigna*species and grown in a semi-arid region (Fery, 2002).

2.1 Physical properties ofbambara groundnut

Bambara groundnut is an annual legume forms pods which grows on or just below the soil surface (Fig 2.1). Table 2.1 presents some of the physical properties of bambara groundnut seeds and other legumes such as cowpea and lentil seeds. The seeds of bambara groundnut vary in terms of size (Length, width and thickness), seed weight, and colour of seeds. However, bambara groundnut seeds possessed different physical properties to other legumes. The seed dimension in terms of length, width and thickness of bambara groundnut seeds showed that it is bigger than the other legumes such as cowpea and lentil.

Bambara groundnut seeds like many other legumes consist of the seed coat and cotyledon as major structural parts (Figure 2.2). The percentage of seed coat in bambara groundnut seeds has been reported to be in the range of 9 to 14% of the whole seeds (Kaptso et al., 2008). The seed coat of bambara groundnut have smooth surfaces and could be cream, brown and red, mottled, with or without hilum colouration (Swanevelder, 1998). Generally, the cotyledon on the other hand forms the larger part of legume seeds. Bambara groundnut seed is made up of two cotyledons enclosed by the seed coat and serves as a storage site for nutrients (Figure 2.2).

2.2 Chemical composition of bambara groundnut seeds

Table 2.2 shows the proximate composition of the bambara groundnut, showing that the seeds is an important source of proteins and carbohydrates and being the major constituents.

The protein content of the seeds is about 17-30%. It has a good balance of both essential and non-essential amino acids (Table 2.3), especially lysine (2.9- 6.8% dry basis) and methionine (1.3- 1.8% dry basis) of seeds (Adebowale et al., 2011; Aremu et al., 2006; Apata & Ologhobo, 1994).

Figure 2.1Bambara groundnut pods (A) and different seed types varying in colour (B) (Swanevelder, 1998)

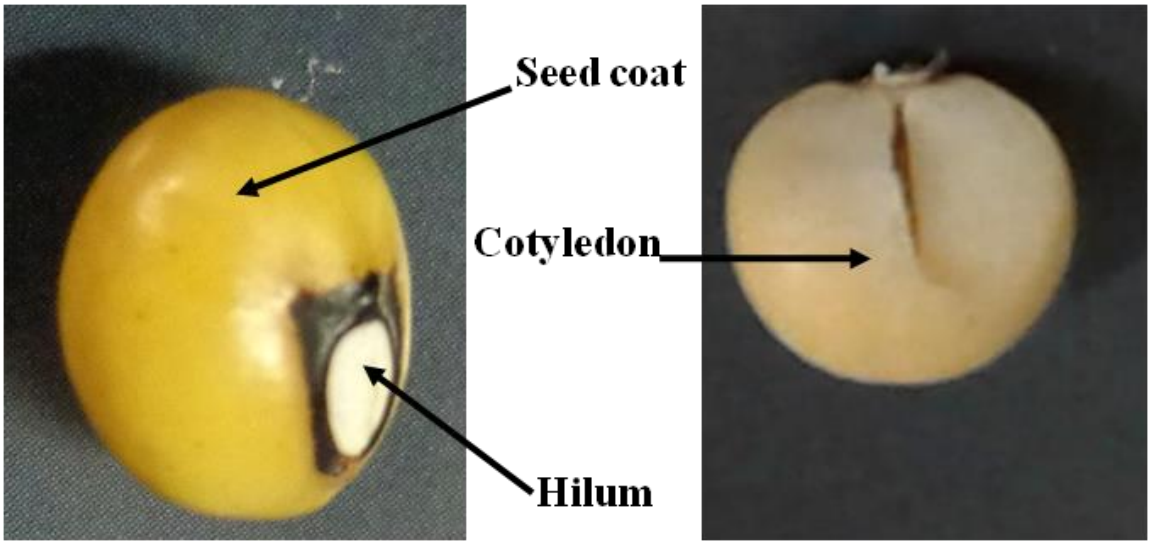


Figure 2.2 Morphology of the bambara groundnut seed showing seed coat, hilum and cotyledon

Table 2.1 Seed characteristics of bambara groundnut, cowpea and lentil

		Seed characteristics						
Legume seeds	Origin	Length (mm)	Width (mm)	Thickness (mm)	1000-seeds weight (kg)	% seed coat	Diameter (mm)	Surface colour
Bambara groundnut[1,2,3]	Ghana Cameroon Botswana	10.5 – 14.6	9.4 – 11.6	8.5 – 10.9	0.5 – 0.8	9.2 – 13.6	10.2- 10.5	Cream red black and speckled
Cowpea[2,4]	a	5.5-9.7	3.8- 6.9	5.5- 9.2	0.01-0.2	a	5.4- 7.3	a
Lentil[5,6,7, 8]	Canada Turkey	a	1.7- 5.6	2.4- 2.6	0.04- 0.07	a	6.6- 6.7	Green and red

[a]Not specified, ([1]Baryeh, 2001; [2]Kaptso et al., 2008; [3]Mpotokwane et al., 2008; [4]Kabas et al., 2007; [5]Carman, 1996; [6]Szot et al., 2003; [7]Isik, 2007a; [8]Isik, 2007b)1000 seeds weight

Bambara groundnut like other legumes contains globulin (water soluble) and albumin (salt soluble) as the most predominant classes of protein (Yagoub & Abdalla, 2007).

In addition, bambara groundnut contain carbohydrates of the range of about 40-80%. Table 2.4 presents different fractionsof carbohydrates of bambara groundnut as reported by different authors. Oyeleke et al. (2012) reported that starch was the pre-dominant carbohydrate in bambara groundnut seeds and other carbohydrate such as reducing sugar, raffinose and stachyose sugars are present in lesser amounts.

Bambara groundnut also contains non starch polysaccharides (NSP) are such as pectin, cellulose, hemicellulose (Tharanathan & Mahadevamma, 2003). Brough and Azam-Ali (1992) reported that of the non-starch polysaccharides (2.56 g/kg dry basis) of bambara groundnut, the ratio of insoluble to soluble fractions was 57:43 and that cellulose accounted for 36% of the total NSP. Pectic substances are complex polysaccharide present in the primary cell wall and middle lamella serving as hydrating agents and cementing materials for the cellulosic network (Muralikrishna & Tharanathan, 1994). The pectin molecule is a linear polymer and composed of α-Dgalacturonic acid linked through 1- and 4- positions with a proportion of the carboxyl groups esterified with methanol (Coultate, 2002). Pectin of bambara groundnut constituted the major portion of the soluble cell wall material and comprise of rhamnose, arabinose, galactose and uronic acids (Brough & Azam-Ali, 1992).

Bambara groundnuts also contain bioactive compounds such as phenolics with antioxidant properties (Diedericks & Jideani, 2014). These bioactive compounds are mainly located in the testa of the seeds (Xu & Chang, 2007). Nyau et al. (2015) employing 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) *in vitro* assays to screen for antioxidant properties in two varieties of bambara groundnut. They found that bambara groundnut possess antioxidant activities common to other legumes such as lentil, common beans and chickpeas. However, there are limited literatures on the bioactive compounds and antioxidant properties of bambara groundnut.

Table 2.2 Proximate composition of bambara groundnut seeds (g/100g dry basis)

Moisture (%)	Carbohydrate (%)	Protein (%)	Crude fibre (%)	Ash (%)	Fat (%)	References
11.3- 11.6	57.9- 61.7	24.0- 25.3	3.4- 3.7	3.6- 3.8	6.9- 6.1	Kaptso et al. (2015)
-	86.0	17.7	3.5	4.2	6.8	Eltayeb et al. (2011)
5.6 -6.8	44.9-46.7	29.5 - 30.3	2.2 -3.0	4.3 -4.5	9.6 -9.7	Adebowale et al. (2011)
7.2	46.5	18.5	-	4.75	-	Okonkwo&Opara (2010)
8.8-9.8	64.5 - 70.5	19.30 - 27.0	-	3.5 - 3.8	4.7 -7.0	Nti (2009)
-	65.0	22.7	3.7	3.8	5.0	Yagoub&Abdalla (2007)
2.1-3.0	73.3 - 73.9	11.1 - 11.6	2.1 - 4.1	3.9 - 4.3	4.1 - 6.7	Aremu et al. (2006)
14.0	55.6	22.1	2.3	3.9	2.0	Fasoyiro et al. (2006)
9.5	67.1	20.7 (0.02)	0.8	0.63	1.24	Adebowale et al. (2002)
7.5 - 12.3	52.9 - 60.8	17.5-21.2	1.8 - 2.1	4.0 - 5.0	7.3 -8.5	Onimawo et al. (1998)
-	63.5	18.3	5.2	4.4	6.6	Amarteifio&Moholo (1998)
7.6-8.9	65- 66	20.6-21.4	3.2-3.4	3.5-3.8	6.4-6.7	Apata&Ologhobo (1993)

(-) Not determined, Mean value (%)

Table 2.3 Amino acid composition of protein in bambara groundnut flour (g/100g dry basis)

Amino acid	Flour	Amino acid	Flour
Essential		Non essential	
Isoleucine	3.1-4.3	Aspartic acid	4.9-12.
Leucine	5.9 -7.8	Glutamic acid	15.1-18.5
Lysine	2.9-6.8	Alanine	3.1-4.7
Methionine	1.3-1.8	Arginine	3.6-8.1
Cystine	0.3-1.5	Glycine	2.3-3.82
Tyrosine	3.2-7.4	Histidine	2.0-3.12
Phenylalanine	3.9 -5.7	Proline	3.2-4.9
Threonine	2.4- 5.1	Serine	2.7-5.7
Tryptophan	1.2-1.3		
Valine	3.2-5.1		

Mean values. Adebowale et al., 2011; Mune et al., 2011; Apata&Ologhobo, 1993

Table 2.4 Carbohydrate fractions of bambara groundnut seeds (dry basis)

Energy (kcal)	Starch (%)	Amylose content g/100g Starch (%)	Reducing sugars (g/100g) whole flour	Soluble carbohydrates (%)
380-520[2,3,4]	39.4 – 56.8[1,5,6]	25.0 - 28.0[1]	0.5 – 1.6[2,6]	5.4[5]

Mean values [1]Kaptso et al., 2015; [2]Poulter, 1981; [3]Nti, 2009; [4]Aremu et al., 2006; [5]Yagoub &Abdalla, 2007; Mune et al., 2011

2.2.1 Microstructure of bambara groundnut seeds

The starch granules of bambara groundnut as observed by different authors under an ordinary light microscope (Enwere & Hung, 1996; Adebowale&Lawal, 2002) and scanning electron microscope (SEM) (Kaptso et al., 2015) showed that it varied in shape, some were somewhat elliptical, spherical or irregular in shape (Fig 2.3). The granules possessed fissures or cracks when viewed (Enwere& Hung, 1996; Adebowale&Lawal, 2002). Kaptso et al. (2015) reported a size range of 6 – 35µm in starch granules in two different bambara groundnut. The starch granules of bambara groundnut were reported to be held together by protein matrix (Enwere & Hung, 1996; Sirivongpaisal, 2008). The protein as observed under SEM showed it has a crystalline structure (Fig. 2.3) (Kaptso et al., 2015).

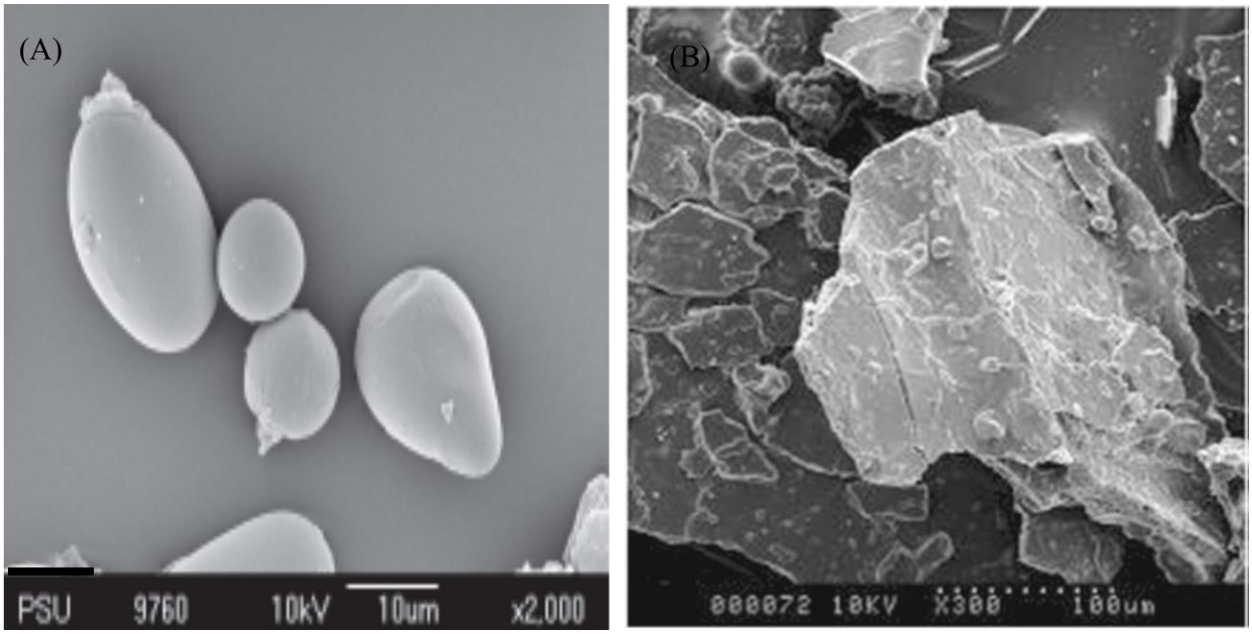


Figure 2.3 Micrographs of bambara groundnut starch granules (A) and protein (B) examined with SEM (bar= 10 µm and 100 µm respectively) (Sirivongpaisal, 2008; Kaptso et al., 2015).

2.3 Cooking time

In general, cooking time is measured relative to the softening of the cotyledon texture (Phadi, 2004; Proctor & Watts, 1987). Table 2.5 presents the cooking time of bambara groundnut and other legume as determined with different methods. As part of limitation of bambara groundnut utilisation, it is clear that bambara groundnut seeds takes a long time of about 3h compared to other legume seeds between 30 min to 2 h. Hard to cook defect contributed to the long cooking of bambara groundnut (Azam-Ali et al., 2001).

However, general practice such as soaking and dehulling wereused with legume to reduce time of cookingto about 12 – 60% because they help in facilitating water uptake in legume seeds (Table 2.5). Several other processing technologies have produced legume with less cooking time. This includes gamma irradiation (Abu &Minnaar, 2009), microwave and autoclaving (Alajaji& El-Adawy, 2006; Mubarak, 2005) and micronisation (Kayitesi et al., 2012; Mwangwela et al., 2006; Arntfield et al., 2001).Micronisation is discussed in detail as it is the focus of the thesis.

Table 2.5 Cooking time of bambara groundnut and other legume seeds using different methods

Legume	Effect of treatment	Cooking time	Measuring Method	Reference
Bambara groundnut.	Soaking (0.1- 0.5% Natural rock salts) at 28 ºC for 3 h.	2 – 3 h Reduced to about 40- 50 min.	Tactile and Hardness tester, Texture analyser.	Annan et al. (2002); Ojimelukwe (1998).
Cowpea.	Soaking (25- 75 ºC) between 12 min – 24 h.	30- 120 min Reduced by 12- 40%.	Tactile, Texture analyser and Mattson bean cooker, Sensory panel	Giami & Okwechime(1993); Sefa- Dedeh et al. (1978); Demooy & Demooy (1990); Taiwo et al. (1997).
Black beans.	Soaking and dehulling.	60-90 min Reduced by 50- 60%.	Mattson bean cooker	Jackson & Varison (1981).
Yellow field peas		15- 24 min.	Tactile, Texture analyser and Mattson bean cooker.	Wang et al. (2003).
Common beans	Soaking (18 h).	30 min- 2 h.	Mattson bean Cooker.	Bernal-lugo et al. (1996).
White gram, Chickpea, Kidney beans and lentil.	Soaking (2-6 h)	13 - 110 min Reduced by 18- 50%	Tactile.	Huma et al. (2008).
Black beans.	Soaking at 22 ºC (12 h).	60 min.	Texture analyser Sensory panel.	Silva et al. (1981).
Dry bean.	Soaking (45 ºC) for 4 h.	92 min.	Mattson bean cooker.	Abdul-kadir et al. (1990).

2.4 Micronisation

Micronisationis the use of infrared heat radiation technology to process moisture conditioned foods. The infrared radiation involves the transfer of thermal energy inform of electromagnetic (EM) waves. It encompasses portion of the electromagnetic spectrum between visible light and microwaves (Fig 2.4). It can be classified into 3 regions based on their corresponding spectral ranges, namely, near-infrared (0.75 to 1.4μm), mid-infrared (1.4 to 3 μm), and far-infrared (3 to 1000 μm) (Sakai & Hanzawa, 1994). Far infrared radiation is useful for food processing because most food components such as starch, protein and pectin absorb energy in the FIR region (Sandu, 1986).

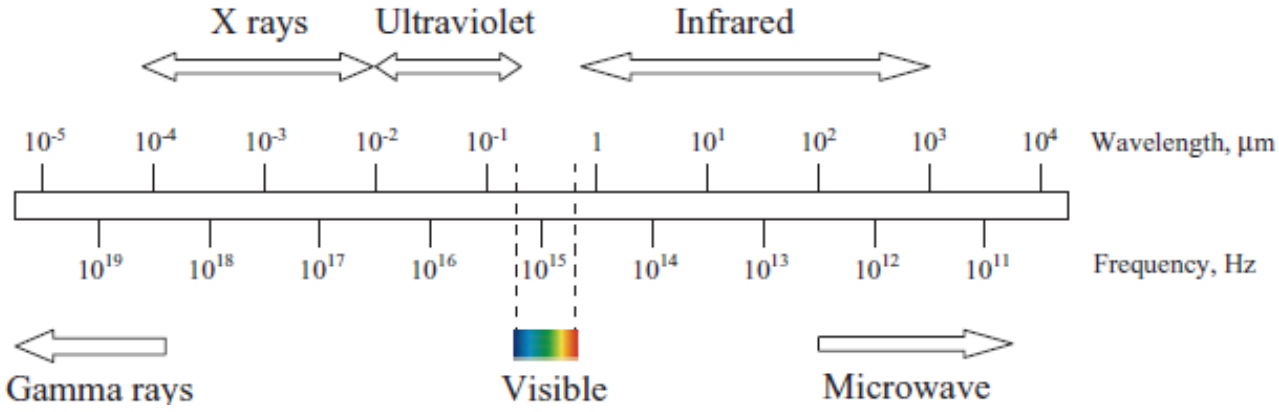


Figure 2.4 Electromagnetic wave spectrum (Khrishnamurthy et al., 2008)

2.4.1 Mode of operation and classification

Figure 2.5 shows the diagram of a laboratory-scale infrared emitter. It consists of two basic components: a vibrating trough, and a set of infrared radiation emitters. The vibrating trough transfers the granular productto infrared heat (Zhao, 2000; Zhenget al., 1998). The infrared radiation emitters generate the infrared radiation energy transferred to the granular products. They are classified based on the wavelength of maximum radiation (Zhao, 2000). Short wave radiation generates temperature above 1500 ºC with maximum radiation at about 1.3μm (Ginzburg, 1969). Long wave generates temperature ranges between 350 ºC and 400 ºC at wavelength of about 3.0 μm (Van Zuilichemet al., 1986).

There are two conventional types of infrared radiators used for process heating namely; electric and gas-fired heaters with temperature range of about 330-2200 ºC. The processing efficiency of infrared radiators depends on the intensity of the radiant energy from the source of radiation. The infrared heating process depends on the distance between the heat source and the product, the presence of water vapor in the air, and the length of the processing time

(Cenkowski et al., 2003). An example of electric infrared radiator is a tabletopmicroniserwith three (2 kW/lamp) Phillips IR lamps which has been used to generate surface temperature between 130 and 170 ºC (Mwangwela, 2006).

2.4.2 Mechanism of micronisation

Micronisation involves simultaneous heat and mass transfer, permitting complex chemical changes during processing (Cenkowski et al., 2003). Figure 2.6 presents a schematic diagram which illustrates the mechanism of the infrared technology with moisture conditioned food (Sakai & Hanzawa, 1994). When moisture conditioned food is exposed to electromagnetic radiation with a wave length range of 1.8–3.4 µm (Fasina et al., 1999), efficiently high to generate high temperatures (750-930 ºC) in a very short time (Sharma, 2009). Theinfrared energy generated is passed into the moisture conditioned food materials (Khrishnamurthy et al., 2008). The energy is absorbed by the moisture conditioned food materials which causes water molecules to vibrates at a frequency of 8.8×10^7–1.7×10^8 MHz (Fasina et al., 2001). During the vibration, intermolecular frictions among the water molecules and other constituents such as starch and protein occurs which result in heat generation and a rise in water vapour pressure in the food (Sharma, 2009). According to Mwangwelaet al. (2006), the rapid temperature increase in pre-conditioned seeds most likely cause liquid water to turn into steam (gas). If the seed is able to hold the expanding gas, then pressure builds up and the cotyledon collapses under this pressure or explodes to release the pressure, resulting in changes of the biomoleculessuch as starch, protein and pectin and resulting changes in their microstructure which modified food materials.

Water is important in the infrared radiation heating of food materials because it absorbs the energy more than other food components because of its dielectric property (Michael et al., 1997). As reviewed byKhrishnamurthy et al. (2008) water and food components absorbed infrared energy most efficiently at different wavelengths of about 2.5 to 1000 µm (FIR) and generate heat through molecular vibration and intermolecular friction of water molecules. The absorption spectral of food components overlaps with one another, while the water effect on absorption of incident radiation is predominant over all the wavelengths because of its dielectric property.

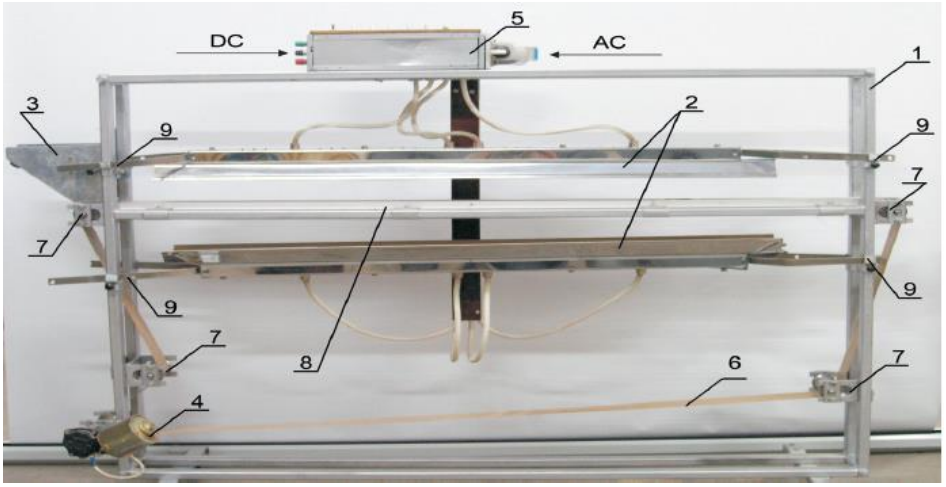


Figure 2.5 Schematic diagram of laboratory-scale infrared emitter ECS-1 type (400W) (Adrejko et al., 2008).
1-Frame, 2- infrared radiator, 3- feeding tank, 4- electric motor, 5- control unit, 6- conveyor belt, 7- rollers, 8- heating zone, 9- adjustment of head position

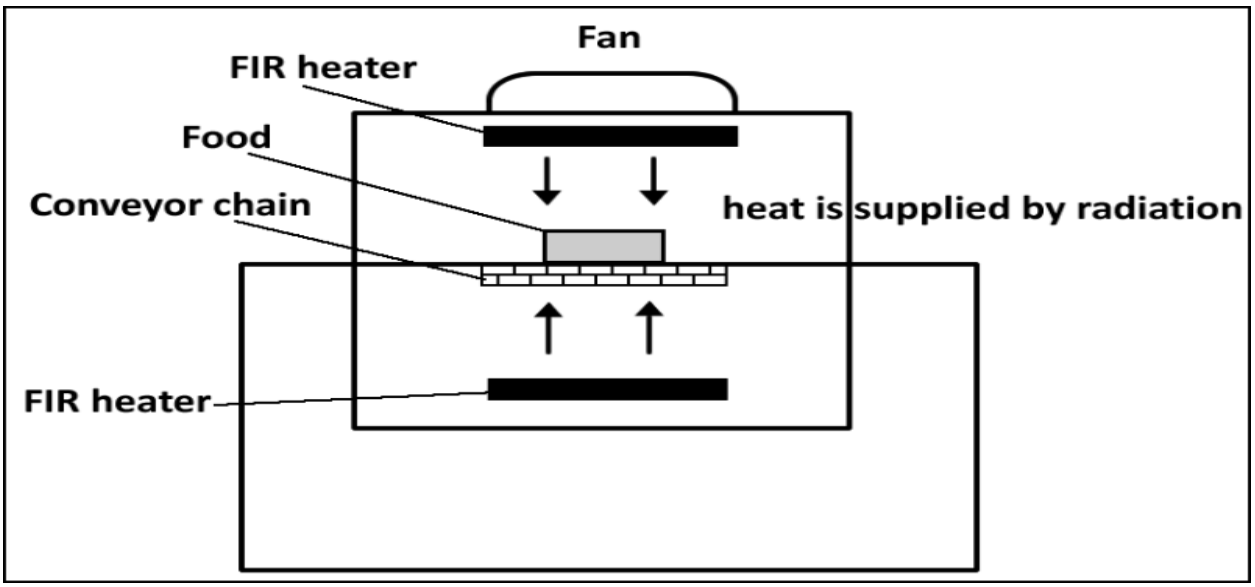


Figure 2.6 Schematic diagram of mechanism of infrared technology (Sakai & Hanzawa, 1994)

2.4.3 Application of Infrared radiation heating in Food processing

The application of infrared radiation heating to food processing has gained more use in different heating processes such as drying, dehydration, blanching, sterilization, roasting, frying and cooking of food products such as potatoes, onion apples, carrots banana, herbs and red pepper, as well as enzymes and pathogen inactivation (Khrishnamurthy et al., 2008).

In general, different factors have been highlighted in the use of infrared radiation heating with food material that influenced the time, rates and efficacy of the processes in their

processed foods (Khrishnamurthy et al., 2008). These factors includes size or thickness of the food samples, surface temperature of infrared heating source, plate distance of infrared heating source, infrared heating source power level, air velocity, moisture content of food samples, peak and bandwidth of wavelength of infrared heating source.

2.4.3.1 Micronisation (Infrared heating) of moisture conditioned legume seeds

In addition to the application of infrared heating with the above listed food products, its awareness in improving the hydration, palatability, cooking time, functionality and digestibility of grains such as legumes in recent years has generated increased interest in its application (Deepa & Hebbar, 2016). Micronisation has been used to improve the cooking quality of legumes such as cowpea, lentil, split pea, and kidney bean seeds. Table 2.6 presents the summary of reports on micronisation of some of the legumes and its quality attributes as reported by different authors. However the information about the effect of micronisation on cooking quality of bambara groundnut seeds is still not known.

Thefactors influencing the efficacy of application of micronisation with legume seeds have also been highlighted by different researchers. Specifically, factors such as seed size, moisture, and surface temperature relatively to the application of micronisation with the legume processing are discussed below because of their importance in the cooking quality of the legume seeds.

2.4.3.1.1 Factors affecting micronisation (infrared heating)

Seed sizes

Seed size is one of the factors influencing the efficacy of micronisation in reducing cooking time of legume seeds. This depends on the type of legume as their response to the infrared heating will varies due to inherent structural differences (Fasina et al., 2001). Cenkowski, Arntfield, and Scanlon (2007) reported that the thickness of a material could influence the depth of penetration of infrared radiation energy through the material.

As a results, Fasina et al. (2001) reported that small legume sized seeds (lentils and green peas) had more reduction in physicochemical property such as protein solubility than large legume such as kidney and pinto beans. This would imply small legume size seeds with high surface area to volume are more uniformly micronised compared to large legumes where thetreatment would be effective more on the outer surfaces than the inside (Sarantinos & Black, 1996).

Table 2.6 Summary of reports on the micronisation of legumes and its quality attributes

Sample	Micronisation conditions	Major Observations	References
Cowpea, lentil and other legumes.	10- 40% moisture Surface temperatures 115 and 170 ºC.	Solubilises pectin.	Arntfield et al. (2001); Ndungu et al. (2012).
		Gelatinised starch: loss of birefringence, Increased enzyme digestion and reduction in enthalpies.	Mwangwela et al., (2007 a).
		Reduced nitrogen solubility IR processing conditions of 10- 41% moisture level for legumes minimises the protein denaturation.	Zheng et al. (1998); Mwangwela et al. (2007b).
Black beans and chickpeas.	17% moisture Surface temperature 69-107 ºC.	Increased water absorption (50%), 16- 64% increased in cooking time and 15- 19% increased in compression force campared to Unmicronised.	Abdul-Kadir et al. (1990); Sarantinos & Black (1996).
Cowpea Varieties.	Tempering: 41%moisture 6 min Surface temperature: 153 ºC.	Increased water absorption. Reduced cooking time by 36 – 47% compared to unmicronised.	Mwangwela et al. (2006).
	Tempering: 41% moisture Surface temperature: 153 ºC.	Reduced cooking time by 50% Affects sensory properties by increasing roasted aroma/flavour.	Kayitesi et al. (2012).
	Tempering: 41% moisture 3 , 6 and 8 min Surface temperature: 130, 153 and 170 ºC.	Micronisation reduced the pasting viscosities of the resulting flours from pre-conditioned micronised cowpea. Fours and starch from cowpea seeds processed at high temperature (170 ºC) exhibited decline in most of the functional properties.	Mwangwela et al. (2007 a). Mwangwela et al. (2007 b).
Cowpea, soybeans, pea and kidney beans.	Tempering: 18-41% moisture 2.5- 12 min Surface temperature: 90-160 ºC.	IR processing improved protein quality and reduced level of antinutrients such as trypin inhibitors urease, oligosaccharides and bioactive compounds.	Vilakati et al. (2015); Khattab & Arntfield (2009); Khattab et al. (2009); Wiriyaumpaiwong et al. (2004); Kayitesi (2013).
Lentils.	Tempering: 29, 33% moisture 100 s.	Micronisation at higher moisture levels reduced lentil hardness. Lentils processed to internal temperature of about 138 ºC exhibited better quality attributes.	Arntfield et al. (1997).
	Tempering: 33% moisture 170 s Internal temperature: 138 and 170 ºC.		Arntfield et al. (2001).
Navy beans, Black beans.	Tempering: 28% moisture, 12 h, 26%, 32 h with water and salt solutions.	Tempering with water prior to micronisation is effective in reducing hardness of beans compared to salts, acid mixtures and EDTA.	Bellido et al. (2006).
Peas.	10% moisture 90 s Temperature; 180 ºC.	Significant shortening of the boiling time of peas, IR-treated seeds had lower compressive strength.	Adrejko et al. (2008).

Moisture

Moisture of seeds prior to the micronisation is another important factor which can influence the efficacy of the process.The moisture conditioning ranging between 17 (low moisture) to 40% (high moisture) prior to micronisation has been reported by different researchers (Table

2.6). High moisture conditioned micronised legumes of about 33-41% (Arntfield et al., 1997; Mwangwela et al., 2006; Kayitesi et al., 2012) had more reductionin the cooking time and hardness than low moisture of about 17% (Abdul-Kadiret al., 1990; Sarantinos & Black, 1996). Cenkowski and Sosulski (1996) found that increase in level of seed moisture (25.8 and 38.6% moisture) prior to micronisation of lentil increased the starch gelatinisation and solubilisation to about 45 to 65% and this was responsible for the reduction in cooking time from 30 min (control) to 15 min (25.8% moisture) and 10 min (38.6% moisture).

Surface temperature

Micronisation temperature also influences the efficacy of the micronisation on seeds cooking properties. Micronisation of pre-conditioned seeds at about 130 and 153 ºC have been reported effective in reducing cooking time of cowpea seed.However, a high micronisation temperature of 170 ºC was reported to be less effective in reducing cooking time despite the high moisture pre-conditioning of cowpea (Mwangwelaet al., 2006). This was reported to be due to protein-protein, protein-carbohydrate cross-linking and amylose associations in the cotyledon (Mwangwelaet al., 2006). Arntfieldet al. (2001) reported an increase in compression force (force required to cut through seeds) of tempered (33% moisture) lentils as the micronisation temperature increased from 138 to 170 ºC. The authors reported that micronised lentils (170 ºC) with substantial less moisture content had a hard texture compared to the micronised lentils (130 ºC) that had high moisture after treatment. The reduction in moisture at 170 ºC could be due to water removal from collapsed capillaries within the seed resulting in a slower rehydration process; this could lead to longer cooking times and consequently higher firmness of the seeds.

Other factors

Among other factors that have not been reported in the literatures which are important to the efficacy of micronisation in influencing cooking properties of legume seeds perhaps is dehulling of the legume seeds. This is because the seed coat of legume seeds contains components such as non-starch polysaccharides (dietary fiber) which were reported high as compared to the cotyledon (Wang et al., 2009). With the presence of the seed coat the infrared radiation energycan only go a little bit inside of the whole grain and almost completely absorbed at seeds surface because of thick layer. Whereas, when the seed coat is removed the thickness of the seeds is reduced and the infrared radiation energy can go more into the cotyledon. Penetration of NIR energy (0.75- 1.4 µm) into different food products

such as bread, biscuit, potatoes, wheat grain and carrots have indicated reduction in thickness of food product increase the penetration of NIR energy (Khrishnamurthy et al., 2008).

2.5 Effect of micronisation on cooking characteristics of pre-conditioned legume seeds

Micronisation of pre-conditioned legume mainly causes change in molecular and microstructural properties of the legume seeds. These structural changes contributed to improvement in the water uptakes, softening and cooking quality of the seeds.

2.5.1 Physicochemical (molecular) changes in micronised pre-conditioned legumes

Micronisation of pre-conditioned legumes causes chemical changes on the major biomolecules such as starch, protein and pectin of the seeds. These changes are discussed below as reported on different legume seeds.

2.5.1.1 Starch

Starch pre-gelatinisation has been reported in pre-conditioned (7-33%) lentils (Arntfield et al., 2001; Arntfield et al., 1997) and other legumes (Fasina et al., 2001) micronised at different temperatures of about 138 to 170 ºC. Arntfield et al. (1997) reported lentils pre-conditioned to higher moisture content (12%) during micronisation have more gelatinised starch than low moisture (9%). High moisture was said to be critical since excess water is necessary for swelling of the starch granule during heat processing. In addition, micronisation caused partial starch gelatinisation in tempered Navy (28%) and black beans (26%) for 32 h with water alone and also in different reagents such as mixture of salts of bicarbonate, carbonates and phosphates, mixture of citric and ascorbic acids, or ethylenediaminetetra-acetic acid (EDTA). The use of different reagents however, did not increase the degree of gelatinised starch in either navy or black beans. The starch pre-gelatinisation (change in crystalline order and nature of the starch granules) reported in pre-conditioned (41% moisture) and micronised (130, 153 and 170 ºC) cowpeas was responsible for the increased water hydration, softening and increased susceptibility of the cowpea starch to α-amylase digestion (68.2– 85.8%) (Mwangwela et al., 2007a).

2.5.1.2 Protein

Protein denaturation has also been reported to occur during the micronisation of pre-conditioned legume seeds. Cenkowski and sosulski (1998) reported that micronisationcauses denaturation of protein in pre-conditioned (26%) split peas. According to Arntfieldet al. (1997), the denaturation of protein in legume was responsible for its loss of solubility. The

authors reported that higher moisture contents during micronisation makes proteins more susceptible to denaturation and aggregation, thereby lowering solubility. Zheng et al. (1998) reported a decrease in nitrogen solubility of different micronised legume seeds in water at pH 6, NaCl salts (0.5 M) and 70% ethanol to unmicronised seeds, indicating protein denaturation (albumins and globulins) in micronised legume seed samples.

Similarly, Fasina et al. (2001) reported lower solubility in different pre-conditioned micronisedlegume seeds than the control sample at different pH levels (pH 2-10). The reduction in the hydrophilicity of legume proteins was as a result of the unfolding of protein molecules, thereby exposing hydrophobic sites (Zheng et al., 1999). The protein solubility of unmicronised cowpeas within the range of 85-88% was reduced to 33-34% respectively (Mwangwela et al., 2007b). Bellido et al. (2006) reported lower soluble protein levels in micronised navy (28%) and black beans (26%) tempered for 32 h using water, mixture of salts of bicarbonate, carbonates and phosphates, mixture of citric and ascorbic acids, or ethylenediaminetetra acetic acid (EDTA). An increasein moisture content of about 26 and 28% (Bellido et al., 2006) and micronisationsurface temperature (138 and 170 ºC) of pre-conditioned lentil progressively increased the reduction in the nitrogen solubility of legumes such as lentils, navy and black beans (Artnfield et al., 2001).

2.5.1.3 **Pectin**

Micronisation has also been reported to cause solubilisation of pectin in moisture conditioned legumes. Arntfieldet al. (2001) reported a decreased level of soluble pectin of pre-conditioned lentils micronised at different temperatures. Also, an increase in soluble pectin content has been reported in pre-conditioned micronised cowpea compared to the unmicronised samples (Ndungu et al., 2012). Pectin solubilityinitiates the disintegration of the middle lamella and cell separation during the cooking of micronised whole cowpea seeds (Mwangwela et al., 2006). Solubilisation of the pectin substances in the middle lamella into water soluble fractions (Ndungu et al., 2012); contributed to shorter cooking timeof legume (Mwangwela et al., 2006). However, Arntfield et al. (2001) reported that non-significant increase observed in soluble pectin in lentils micronised to 138 and 170 ºC, may not be responsible for the reduction in cooking time and changes in texture.

2.5.2 Microstructural changes in micronised pre-conditioned legumes

The structural changes in the biomolecules such as starch, protein and pectinas a result of the micronisation of pre-conditioned legume seeds at molecular level relatively lead to

microstructural change in the seed structure. For example, solubilisation of pectin may be responsible for the separation of parenchyma cells and starch pre-gelatinisation responsible for the deformation of starch granular structure in the cotyledon of pre-conditioned micronised legume seeds (Mwangwela et al., 2006). The rapid pressure build up and evaporisation of water during micronisationhas been reported to cause the cotyledon to ruptured thus leading to the occurrence of fissures on the surface structure of the seed coat and the creation of cavities in cotyledonof pre-conditioned micronised lentils (33%, 170 ºC) (Arntfield et al., 2001) and cowpea (41%, 153 ºC) (Mwangwela et al., 2006) (Figure 2.7). The cell walls of pre-conditioned micronisedlentils were also reported to be more susceptible to fractures exposing the starch granules and opening up the microstructure of cotyledon of lentil (Arntfield et al., 2001; Scalon et al., 1999). Micronisation has been shown to alter the molecular structure of starch of pre-conditioned cowpea, as evidenced by loss of birefringence (Mwangwela et al., 2007b). Mwangwela et al. (2006) reported micronisation causeddisruptions in the middle lamella, loosening of the parenchyma cells, increased intercellular space of the cells and possible cell separation in pre-conditioned micronised cowpea seeds (Figure 2.8 (a), (b) and (c)).

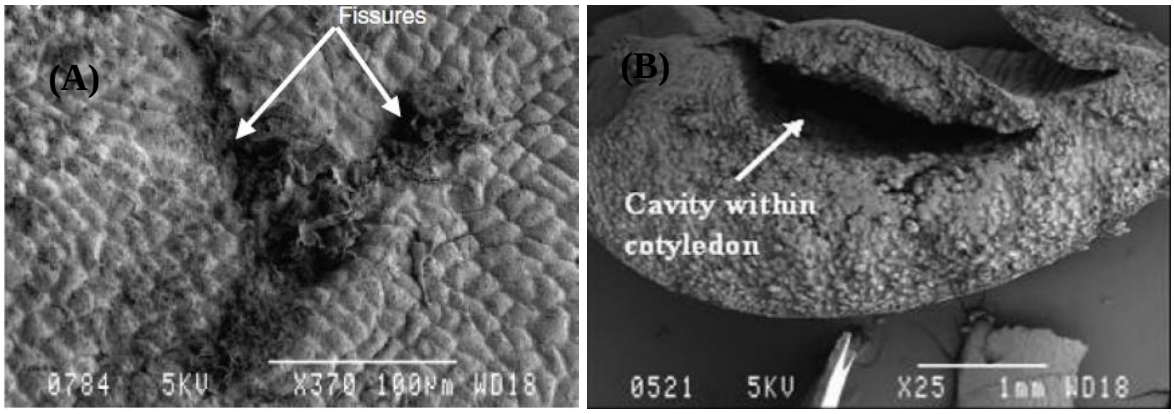


Figure 2.7 Fissures on the seed coat (A) and formation of cavities on the cotyledon (B) of pre-conditioned micronised cowpea seeds (Mwangwela et al., 2006)

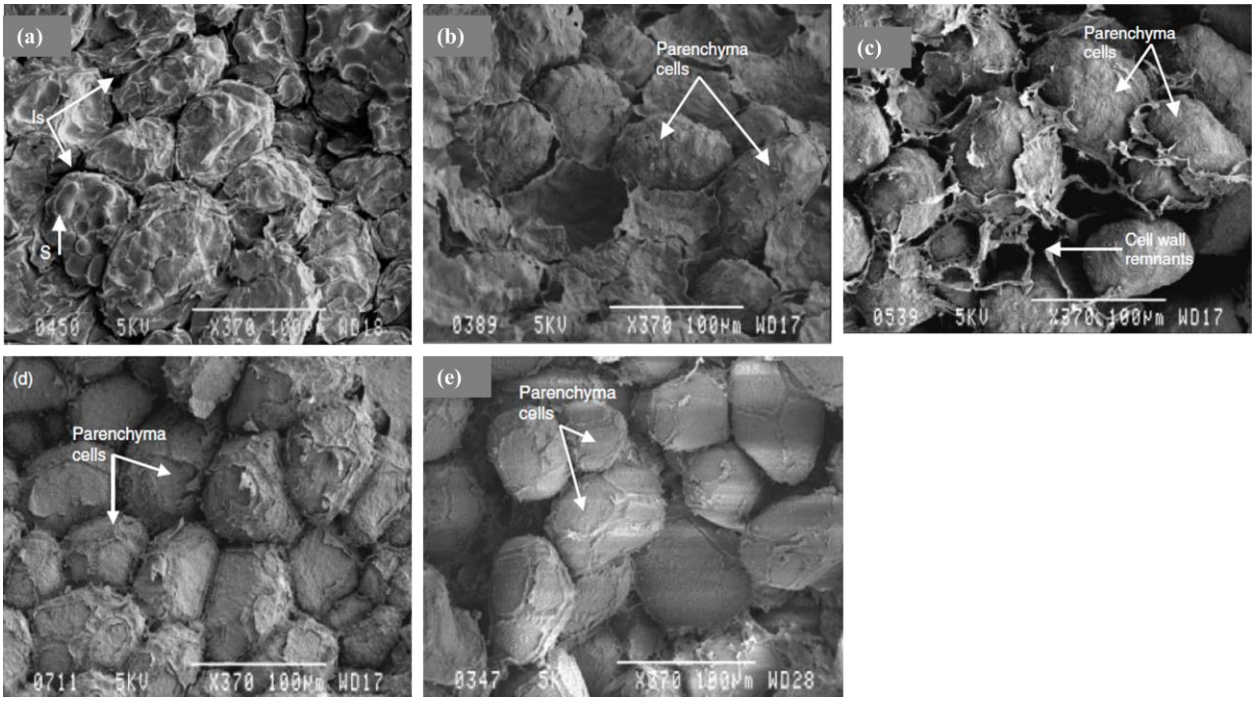


Figure 2.8 Effects of micronisation on cotyledon structure of preconditioned cowpea seeds (Mwangwela et al., 2006).

Cotyledon cross-sections of: (a) raw (b) micronised (c) micronised and soaked (d) fully cooked unmicronised at 60min (e) fully cooked micronised at 30min. Is, intercellular space; S, starch granule.

2.5.3 Hydration properties

Micronisation has been reported to improve water uptakes of moisture pre-conditioned seeds during soaking. Abdul-kadir et al. (1990) reported that micronisation (17% moisture, 99ºC and 107 ºC) improved water uptake and increased the degree of swelling of pinto beans during 8h of soaking (22 ºC). Similarly, higher water uptakes were reported in micronised pre-conditioned (41%) cowpea (Mwangwelaet al., 2007a;Mwangwela et al.,2006) and other legumes such as green pea, kidney, black and pinto beans and lentils (7-10%) during 6h soaking (22 ºC) (Fasinaet al., 2001). This is because in unmicronised cowpeas the seed coat is the first barrier to water transfer into the cotyledon and water can only have access through the micropyle (Penicela, 2011). However, the seed coat is no longer intact in micronised seeds (Figure 2.8). Therefore, water access increases into the cotyledon through the fissures and cavities createdin the seed coat.

In addition, Abdul-Kadir et al. (1990) found that the hydration capacity of micronised pinto beans after 8h soaking (22 ºC) were twice that of the raw pinto seeds. However, the hydration capacity of pre-conditioned and micronised cowpea seeds after 18 h of soaking (22 ºC) reduced by 20–25% compared to unmicronised cowpeas (Mwangwela et al., 2006). Fasina et

al. (1999) also reported reductions in the hydration capacity of soaked micronised barley stated that the shrinkage of stomata like cells could be responsible for the occurrence.

Micronisation has also been reported to improve water uptakes of pre-conditioned seeds during cooking. Cenkowski and Sosulski (1997) found rapid improvements in the water uptake of micronised (386 gkg^{-1} moisture, 150 ºC) lentils during cooking. At 15min, when micronised seeds were fully cooked (starch fully gelatinised), water uptake was about 60%; the control reached 60% and was at the fully cooked stage in 30min. Similarly, the water hydration in micronised split pea (11% moisture) seeds increased by 7% more than the control during cooking over 15 min periods and the maximum water uptake after 15min was more than 50% (Cenkowski & Sosulski, 1998). The increase in hydration of micronised lentils (17-45%**)** was reported to be due to the hydrophilic nature of the gelatinised starch permitting faster absorption of moisture (Scanlon et al., 2005). Andrejko et al. (2008) reported the water hydration of pre-conditioned (10% moisture) micronised (180 ºC) pea increased by 1.8 times than the unmicronised pea during cooking over 30 min, possibly due to reduction in bulk density and the development of fissures. Micronisation altered the granular structure of cowpea starch, thereby requiring less time to hydrate and disrupt the remaining starch structure during subsequent cooking process of the micronised seeds (Mwangwela et al., 2006).Water absorption increased during the cooking of the micronised cowpeas (Mwangwela et al., 2006) and water uptake had a significant negative correlation with the texture of the cowpeas (Arntfieldet al., 2001).

2.5.4 Cooking time

In table 2.6, micronisation has been reported to reduce the cooking time of different legume seeds using various conditions. It was clear that legume seeds pre-conditioned at about 40% had percentage reduction of about 30- 50% in their cooking time. The reduction in compression force of cooked micronised seeds was reported as an indication of a soft texture as compared the unmicronisedseeds. The reduction in the cooking was associated to the rapid improvement in the hydration during cooking. The rapid water hydrations into the micronised seeds shorten cooking time and led to soft texture of different micronised grains compared to the unmicronised (Fasina et al., 2001). In relation of structural changes in cowpea seeds to cooking time, Mwangwela et al (2006) reported similarity in microstructure of fully cooked unmicronised cowpeas at 60 min and micronised cowpeas at 30 min cooking time (Figure 2.8 (d) & (e)).

On the other hand, with limited initial moisture the increase in cooking time of some micronised legumes was due to possible hardening (Andrejko et al., 2008). This is because high moisture in the seed is critical since moisture is necessary for the removal of amylose and swelling of the granule during gelatinisation (Hood, 1982).

2.5.5 Functional properties of resulting flours

The functionality of legume flour has been related to its biomolecules. Foaming, emulsification, and water and oil absorption has been closely related to the function of proteins (Aremu et al., 2007); viscosity and swelling characteristics related to starch (Singh et al., 2004); and gelation characteristics to both starch and proteins (Prinyawiwatkul et al., 1997). Bambara groundnut has good functional properties such as water absorption, water binding, foaming, gelation, bulk density and emulsification (Eltayeb et al., 2011; Aremu et al., 2007; Onimawo et al., 1998) and it has been considered to have great potential to be incorporated into various human foods (Onimawo et al., 1998).

Various studies reported the effect of micronisation of moisture conditioned seeds on functional properties of resulting flours of cowpea (Mwangwela et al., 2007a; Mwangwela et al., 2007b) and split peas (Cenkowski & Sosulski, 1998) such as water absorption capacity, swelling power, water solubility, foaming capacity, gelation and pasting viscosity. However, the information on the effects of micronisation on functionality of bambara groundnut flour is still not known.The impact of micronisation on the resulting flours was attributed to the major molecular changes on starch and protein that occurred during treatment of the seeds (Mwangwela et al., 2007a; Mwangwela et al., 2007b). The mechanism responsible for the molecular changes in these biomolecules which are starch and protein are explained as above under the physicochemical changes in micronised pre-conditioned legumes. Therefore, the functional properties of flours alternative legumes as related to this research were discussed below.

Water absorption

Micronisation of pre-conditioned cowpea (41% moisture, 130 and 170ºC) (Mwangwela et al., 2007b), splits peas (Cenkowski & Sosulski, 1998) and other legumes (<10% moisture, 140 ºC) such as green pea, kidney, black and pinto beans and lentil (Fasina et al., 2001) was reported to increase the water absorption of the resulting flours. This was due to modification of starch and protein which were important constituents that determine water absorption properties of heterogeneous systems such as flour (Mwangwela et al., 2007b).Vilakati et al.

(2015) found that micronisation increase the water absorption indexof resulting flours of cowpea by 1.7-2.4 times as compared the unmicronised due to the changes in cellular structure and starch gelatinisation.

Swelling and water solubility index

Micronisation of pre-conditioned cowpea at 130 and 170 ºC reduced the swellingindex of the resulting flours by 17.8% and 18.2% respectively andthe swelling index had a negative correlation with the water absorptionof the flour (Mwangwela et al., 2007b). Micronisation (130 and 170 ºC) also reduced the water solubility index (WSI) of resulting flours by 42% and 55% respectively and this positively correlated with the NSI of the flour (Mwangwela et al., 2007b).

Pasting properties

Figure 2.10 shows a pasting curve ofthe effect of micronisation (130 ºC and 170 ºC surface temperature, 3and 8min) on pasting properties of cowpea flour from micronised seeds (Mwangwela et al., 2007a). The pasting curve in this study was used to track starch granule swelling and stability in flours from both unmicronised and micronised cowpea seeds. The flour from unmicronised cowpeas exhibited a cold swelling peak characteristic. However, the flour from micronised cowpea (41% moisture, 130 and 170 ºC) (Mwangwela et al., 2007a) split peas (26% moisture, 110 ºC) (Cenkowski & Sosulski 1998) seeds did not give cold swelling peak characteristic as expected in pre-gelatinised starch (Figure 2.10). Contrary to the reduction in the viscosity of paste from flour from micronised cowpeas (Mwangwela et al., 2007a), Cenkowski and Sosulski (1998) reported rapid increase in viscosity of flour from micronised split pea than the unmicronised. Mwangwela et al. (2007a) hypothesized that either starch is been depolymerised or possibly due to reduction in swelling potential of physically induced cross linked starch during subsequent gelatinisation which reduced water access. In contrast, Cenkowski and Sosulski (1998) hypothesized that an increased in starch swelling during pasting may be responsible for the outcome. However, this mechanism is not fully understood.

In addition, an increase in pasting temperatures (onset T_o, peak T_p and endset T_c) were reported in flour of pre-conditioned (41% moisture) and micronised (surface temperature 130 and 170ºC) cowpea seeds and their isolated starch as compared to the unmicronised samples. These were attributed to the starch granule modification in micronised legume samples which reduced water access, presence of other competing hydrophilic molecules such as proteins

and amylose–amylose interactions increased starch granule crystallinity requiring higher temperatures for melting.

2.5.6 Nutritional and health promoting properties of resulting flours

Table 2.6 shows some studies on the effect of micronisation on the nutritional and bioactive properties of flour from legume seeds. Micronisation improved the nutritional quality of the legumes such as cowpea, pea, soybean and kidney beans in terms of protein quality; essential amino acids (Lysine), *in vitro* protein digestibility, reduction of antinutrients such as trypin inhibitors, urease and oligosaccharides. Wiriyaumpaiwong et al. (2004) reported that micronisation (18% moisture, 160 ºC) for about 12 min did not show any significant effect on the lysine content of soybean. However in other studies, the lysine content of flour from micronised cowpea seeds at about 5 min met the lysine requirements of 2-5 year old children (41% moisture, 153 ºC) (Vilakati et al., 2015; Khattab et al., 2009). Khattab et al. (2009) reported that micronisation (24% moisture, 90 ºC) of legumes such as cowpea, kidney bean and pea at about 3 min increased the lysine content from about 6-6.4 g/100g protein to about 7-8 g/100g protein.

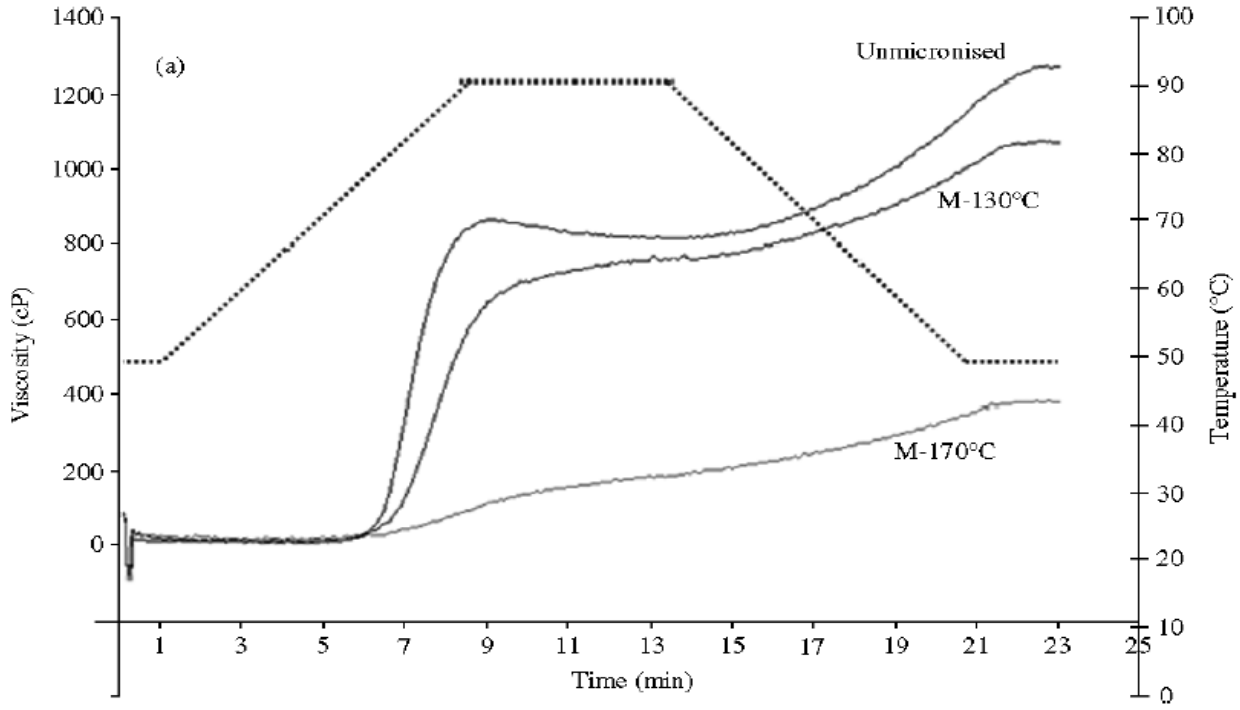


Figure 2.9 Effects of micronisation temperature (130 and 170ºC) on pasting properties of cowpea flour from micronised seeds (Mwangwela et al., 2007a).

Micronisation was reported to improve the *invitro* protein digestibility in legume seeds (Vilakati et al., 2015; Khattab et al., 2009). Vilakati et al. (2015) reported about 3% increase in the protein digestibility of cowpea as a result of the miconisation. The improvement in the digestibility of protein was ascribed to the destruction of some heat labile antinutrients (protease inhibitors) and denaturation of proteins thus increasing protein accessibility to the enzymatic attack during digestion (Khattab et al., 2009). Vilakati et al. (2015) and Khattab & Arntfield (2009) reported about 80 to 90% reduction in trypsin inhibitor in cowpeas. Micronisation (20% moisture, 90 ºC) also reduced oligosaccharides such as stachyose, verbascose and raffinose by about 20% in legumes such as kidney beans, cowpea and pea (Khattab & Arntfield, 2007).

Micronisation influences some health promoting properties of legume. Kayitesi (2013) reported micronisation of cowpea caused a reduction in the total phenolic content of the extracts from the cooked micronised cowpea samples. However the extracts from the cooked micronised cowpea still exhibited radical scavenging properties thus offering potential health benefits.However, the information on the effect of micronisation of legume seeds on the nutritional and bioactive compounds such as phenolic compound and their radical scavenging activities is still limited. Moreover, the information on the effect of micronisation on the nutritional and bioactive propertiesof bambara groundnut flour is still not known.

2.6 Gaps in Knowledge

- Micronisation has reduced the cooking time of smaller whole legume seeds such as cowpea and lentils preconditioned to about 30 – 40 but has not been used with bigger grains such as bambara groundnut seeds.
- Moisture conditioning of whole legume seedsprior to micronisation was achieved mostly in previous studies by tempering technique with limited or no research on pre-soaking and dehulling of legume seeds prior to micronisation.
- The micronisation of pre-conditioned legumeseedscaused reduction in the pasting viscosity of the resulting flours. However, the mechanismresponsible for reduction in the pasting viscosity of the resulting flours has not been elucidated.
- Micronisation of pre-conditioned legume seeds has the potential to produce instant flour with changed nutritional quality. However, the nutritional and health promoting properties of the porridge may depend on the processing conditions of micronisation such as the pre-soaking to high moisture level, dehulling and micronisation time.

3 HYPOTHESIS AND OBJECTIVES

3.1 Hypothesis

1. When pre-soaked bambara groundnut seeds (53% moisture) are micronised to a mild (130°C) final surface temperature, the micronisedbambara groundnut samples will have a shorter cooking time during subsequent cooking than unmicronised seeds. This is because micronisation of the pre-soaked bambara groundnut seeds will cause infrared energy absorption by water and other seeds biomolecules such as starch, protein and pectin (Fasina et al., 2001). Heat generated through molecular vibration and intermolecular frictionof water and biomolecules will turn water to steam and building up pressure within the seeds (Mwangwela et al., 2006). The pressurised superheated steam then transform the biomoleculesinterms of partial gelatinisation of starch, protein denaturation (Mwangwela et al., 2006) and solubilisation of pectin (Ndungu et al., 2012). This enhances fissures on the seed coats and cotyledon; and disintegration of the middle lamella by pectin solubilisation (Mwangwela et al., 2006). These changes facilitate water absorption during soaking and cooking and reduction in the cooking time. Dehulled samples will have more reduction in cooking time than whole because of the seed coat absence, splitting of seeds and higher impact of micronisation on the pre-soaked dehulled seeds than whole.
2. Resulting flour from bambara groundnut seeds pre-soaked (53% moisture) and micronised (130 °C) for 5, 10 and 15 min for whole and dehulled samples will reduce the pasting viscosity as compared to flour from unmicronised pre-soaked (53% moisture). Micronisation will causemolecular changes in starch and protein ofresulting flours from micronised legumes (Mwangwela et al., 2007a). Denatured protein of resulting flours from micronised legumes will be more hydrophobic as the nitrogen solubility index is reduced (Mwangwela et al., 2007b; Zheng et al., 1998). The interactions of partially gelatinised starch and denatured protein matrices (Mwangwela et al., 2007b) will partly preventing starch hydration, dispersion of starch molecules and entanglement during pasting and thus reducing pasting viscosity (Mwangwela et al., 2007b).
3. Cooking the resulting flours from pre-soaked micronised bambara groundnut seeds into soft porridgewill change the nutritional and health promoting properties (total phenolic content and antioxidant activity) of the extracts from cooked soft porridge. This is because cooking of the resulting flours from pre-soaked micronised bambara

groundnut seeds will depolymerise the micronise-induced biomolecules such as starch and protein into lower, more soluble molecular fractions (Vilakati et al., 2015; Kayitesi, 2013). The decrease in viscosityof cooked soft porridgewill increase the digestion of biomolecules such as of starch and protein (Oladiran & Emmambux, 2016). The increase in soluble pectin due to micronisation (Ndungun et al., 2012), will release extractable phenolic compound and antioxidant activity in the cooked soft porridge of whole micronised bambara groundnut samples. However, with the absence of seed coat and micronisation of dehulled bambara groundnut the bioactive properties of the extract from cooked dehulled micronised samples will be lowered. This is because absence of seed coat and heat processingwill causecomplexation of seed phenols with its macromolecules such as proteins thus reducing the extractability of phenolic compounds (Kayitesi, 2013; Awika et al., 2003).

3.2 Objectives

The objectives are to:

a) To determine the effects of micronisation of pre-soaked whole and dehulled bambara groundnut seeds on their cooking characteristics and microstructure in order to reduce the cooking time.
b) To determine the effects of micronisation and dehullingof pre-soaked bambara groundnut on physicochemical, microstructure and functional properties of the resulting flours in order to improve its utilisation of their resultant flours.
c) To determine the effects of micronisation of pre-soaked whole and dehulled bambaragroundnut seeds on the *in vitro* protein and starch digestibility, total extractable phenolics and antioxidant activity and flow behaviour of their cooked samples in order to improve the utilisation of their resultant flours.

4 RESEARCH

The research work comprised three phases. The first research chapter (section 4.1) deals with the effect of micronisation of pre-soaked whole and dehulled bambara groundnut *(Vigna subterranea)* seeds on their cooking characteristics and microstructure. The second chapter (section 4.2) determines the effects of micronisation and dehulling of pre-soaked bambara groundnut seeds on microstructure and functionality of the resulting flour. The final research chapter (section 4.3) determines the flow behavior, nutritional and health promoting properties of cooked soft porridge from pre-soaked micronised bambara groundnutflour.

4.1 Effects of micronisation of pre-soaked whole and dehulled bambara groundnut (*Vigna subterranea*) seeds on their cooking characteristics and microstructure

Abstract

Bambara groundnut (*Vigna subterranean (L.) verdc*) seed is an indigenous legume grown across Africa with good nutritional value in terms of protein quality. However, the cooking time of more than 2 h has limits itsutilisation. Micronisation is an infrared radiation technology and can be used toreduce the cooking time of pre-conditioned whole grain legumes for example cowpea, lentil, and pea. In this study, the effects of pre-soaking bambara groundnut seeds (a bigger grain)and high moisture (53%) soakingwith or without dehulling, followed by micronisation (0, 5, 10 and 15 min) on their cooking characteristics were studied. Micronisation (53% moisture, 130 ºC) causes a significant ($p<0.01$) reductions in 162min cooking time of whole bambara groundnut seeds by 49-62%. It reduced 41 min cooking time of pre-soaked dehulled groundnuts by 7-22%. Micronisation of the pre-soaked bambara groundnut causes microstructural changes in the cotyledon and molecular changes in the components of parenchyma cells of the seeds such as starch, protein, and pectin. A significant ($p<0.001$) increase in soluble pectin with micronisation could enhance cell wall separation. These modifications could rapidly improve water uptakes causing significant ($p<0.01$) increase in their water absorption during 24 h of soaking (25 ºC) and 120 min of cooking (95 ºC). The removal of seed coat seems to play more significant role in water uptakes of pre-soaked micronised dehulled samples especially during cooking. In conclusion, bambara groundnut seeds could be processed to a short time cooking legume demanding less energy and time, thus improving its consumption and utilisation.

4.1.1 Introduction

Bambara groundnut *(Vigna subterranean (L.))* verdc seeds are an indigenous legume grown across the African continent. It can grow under low rainfall, low fertile soil condition and mostly preferred by many indigenous people (Azam-Ali et al., 2001). Kaptso et al. (2014) reported two varieties of bambara groundnut (white and black respectively) seeds nutritionally contain proteins (25.3 and 24%), carbohydrates (57.9 and 61.7%), fat (5.9 and 6.1%) and fibre (3.4 and 3.7%) on the dry basis. In addition, bambara groundnut seeds protein are rich in lysine and leucine with about 6.98 to 9.71% dry basis (Mune et al., 2011). The matured seeds are commonly eaten wet cooked and roasted as part of the diet in Africa. Many authors have mentioned that bambara groundnut seeds require a long cooking time, for example, Ojimelukwe (1999) and Annan et al. (2002) reported the cooking time of about two hours or more. This may hinder its consumption and utilisation, especially with urban consumers whose food choices is based on convenience lifestyle.

Soaking and dehulling are among pre-processing treatment to improve cooking, nutritional and functional qualities of legume (Subuola et al., 2012). Soaking of legume seeds facilitate uptakes of water and enhances the removal of the seed coat from the cotyledon (Kaptso et al., 2008). Dehulling improved quality of different legumes; increased the protein content by 5% and provide a product with appealing appearance (Nti, 2009) and reducing antinutrient such as trypsin inhibitors and phytic acids (Wang et al., 2009; Mubarak, 2005). The use of low concentrations (0.3 and 0.5%) of a locally available natural rock salt (Kawe) in Ghana, for pre-soaking bambara groundnut for six hours has only been reported as means of reducing the two hours cooking times of varieties of bambara groundnuts to about 40 to 50 min (Annan et al., 2002). Information on methods and technology to reduce the cooking time of bambara groundnuts are still limited in the literature compared to other legumes such as cowpea, chickpea, and mung beans. For examples processing technologies employed to reduce the cooking time of other different legumes are the use of gamma irradiation for cowpea (2-10 kGy) (Abu & Minnaar, 2009), microwave and autoclaving for chickpea (Alajaji & El-Adawy, 2006) and mung bean seeds (Mubarak, 2005).

Micronisation technology has been used to reduce cooking time of different legumes such as cowpea (Kayitesi et al., 2012; Mwangwela et al., 2006), lentil (Arntfield et al., 2001) and pea beans (Cenkowski & Sosulski, 1998). It involves the application of an infrared radiation to moisture pre-conditioned food materials. On exposure of food to the near infrared radiation (wavelength range of 0.75 to 1 µm), part of the energy is absorbed and heating results due to molecular vibrational states of water and other biomolecules in the food (Krishnamurthy et

al., 2008). The increase in water vapour pressure and temperature inside of micronised seeds causes structural changes such as physical fissures on seed coat and cotyledon (Mwangwela *et al.*, 2006), starch pre-gelatinisation (Mwangwela et al.,2007a; Cenkowski & Sosulski, 1998), protein denaturation (Mwangwela et al.,2007b; Zheng et al.,1998; Cenkowski & Sosulski, 1998) and pectin solubilisation (Arntfield et al., 1997).

After micronisation of pre-conditioned cowpea (Mwangwela et al., 2006) and lentil (Arntfield et al., 2001) seeds, the improvement in water uptakes of the micronised whole legume seeds both during soaking and cooking are related to the reduction in the cooking time. Micronisation of moisture pre-conditioned whole grain legume seeds at different parameters of about 30- 40% moisture and 130- 170 ºC surface temperature reduced the cooking time of legume such as cowpea (Kayitesi et al., 2012; Mwangwela et al., 2006), lentil (Arntfield et al., 2001) and pea beans (Andrejko et al., 2008) by more than 40%. Scanlon et al. (2005) reported that different moisture level (17- 45%) prior to micronisation brings about different changes in physical, physicochemical and chemical properties of micronised lentil.

Although there are several works on micronisation reducing cooking time of other pre-conditioned legume seeds such as cowpea (Kayitesi et al., 2012; Mwangwela et al., 2006) and lentil seeds (Arntfield et al., 2001), there are limited or no research on soaking of bigger seeds as compared to smaller legume seeds such as cowpea and lentil pre-conditioned to about 40% prior to micronisation. Bambara groundnut seeds are about 8.2-14.9 mm (length), 5.3-12.9 mm (width) and 1.2-12.7 mm (height) (Mpotokwane et al., 2008) compared to cowpeas with size of about 9.2 mm (length), 6.5 mm (width) and 6.0 mm (height) (Kabas et al.,2007) and diameter and thickness of lentil with 6.64 mm and 2.65 mm (Carman, 1996). Since information about micronisation of pre-soaked bigger seeds like bambara groundnut is still lacking in literature as most research were done on legume seeds pre-conditioned to about 40%. This research deals with micronisation of pre-soaked bambara groundnut seeds. Therefore, the objective was to determine the effect of micronisation of pre-soaked whole and dehulled bambara groundnut seeds on their cooking characteristic in order to reduce the cooking time.

4.1.2 Materials and methods

4.1.2.1 Raw materials

Mixed bambara groundnut seeds type was purchased in Mbare produce market, Harare, Zimbabwe. The mixed bambara groundnut seeds type means that it contain seeds of different colours and sizes. The bambara groundnut seeds were sorted according to seed size and colour of seed and colour of the eye. Cream bambara groundnut seeds were categorised based on size differences as small, medium and large types of bambara groundnut seeds (Figure 4.1). Uniform medium cream bambara groundnut seeds with a black eye, with seed weight (77.54 g/100seeds) and seed dimension (Length (1.23 cm × Width (1.01 cm) × Height (0.94 cm) were used in this research. The seeds were thoroughly cleaned and packed in polypropylene bags and stored at 4 ºC until the time of use.

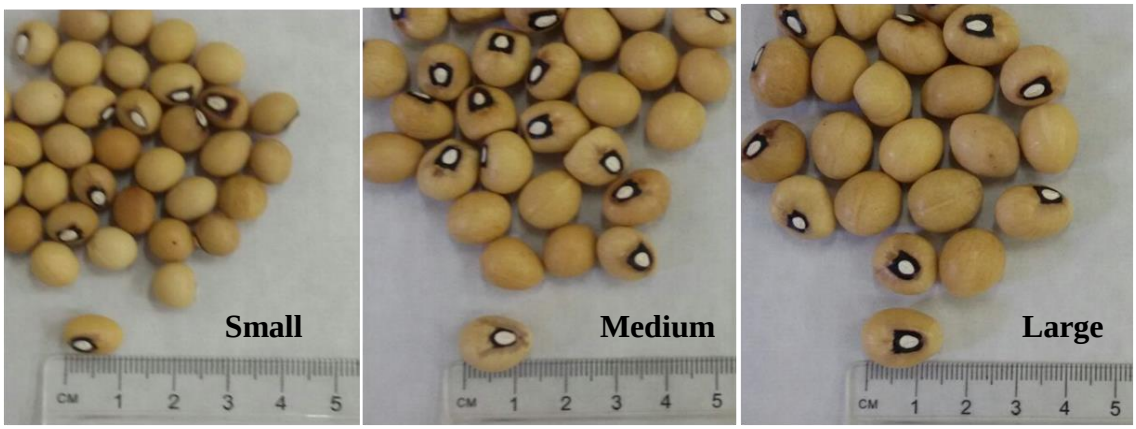


Figure 4.1 Seed dimensions of cream bambara groundnut (small, medium and large).

4.1.2.2 Method

Hydrothermal process

The uniform bambara groundnut seeds (100 g) were soaked for 24 hours at ambient temperature (25 ºC) to achieve 53% moisture level. The pre-soaked bambara seeds were manually dehulled by removing the seed coats for dehulled samples. A table top microniser with three 2 kW Phillips IR lamps (Technilamp Pty, Johannesburg, South Africa) with two of the three lamps used to micronise pre-soaked whole and dehulled bambara groundnut seed samples in rectangular (478 mm diagonal and 15 mm high) aluminium tray (Figure 4.2). To achieve a surface temperature of 130±5 ºC, the distance between the lamps and the bambara groundnut seeds was set at about 250 mm. The microniser was pre-heated for 20 min before the samples were then heated at130 ºC surface temperature for 5, 10 and 15 min. After

micronisation, the pre-soaked micronised bambara groundnut seed samples were dried in a hot air dryer (50 ºC) for 12 h and cooled to room temperature for 1 h with final moisture contents of about 7-11%. The dried pre-soaked micronised bambara groundnut seed samples were packed in an air tight container and kept at 4 ºC. The experiment was repeated three times and then the samples were analysed.

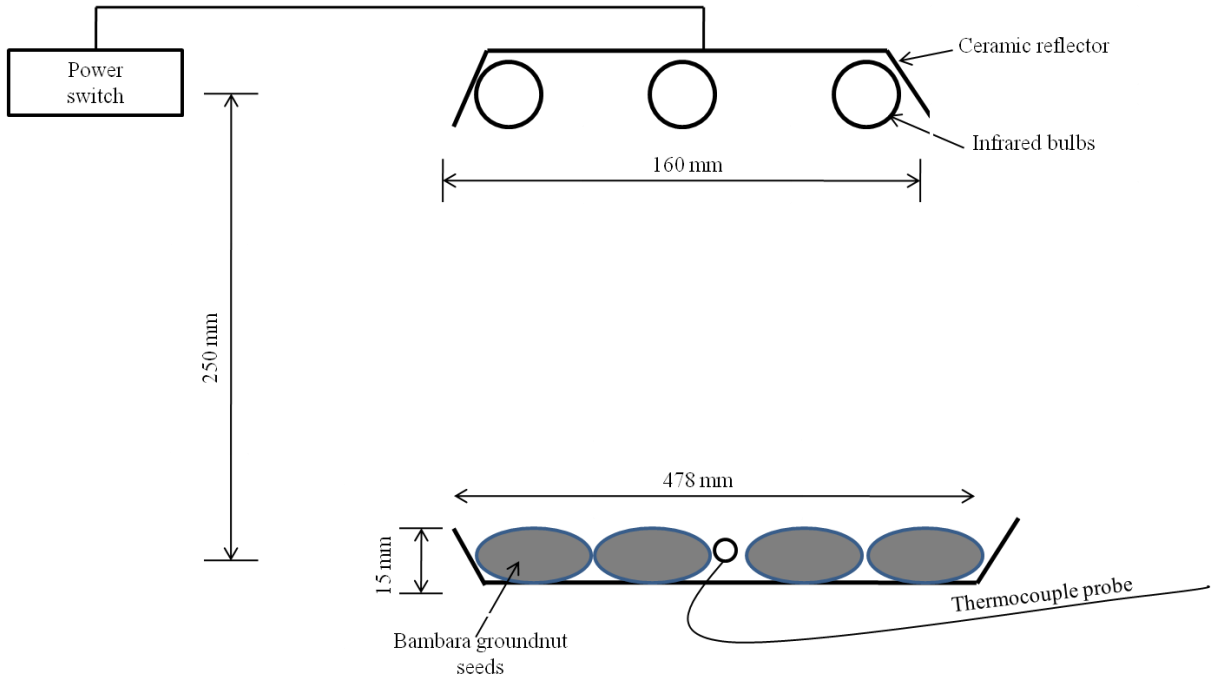


Figure 4.2 Experimental set-up

4.1.2.3 **Analyses**

Moisture content

The moisture contents of unmicronised and micronised bambara groundnut seeds (whole and dehulled) after micronisation and drying were determined according to the method described by Ajibola et al. (2003). Bambara groundnut seed sample was weighed into pre-dried moisture tin. The sample was dried in a hot air oven at 103 ºC for 72 h to evaporate the moisture, cooled in desiccators and weighed.

Cooking time

The cooking time of unmicronised and micronised bambara groundnut seed sample was determined with a Mattson bean cooker (Mwangwela et al., 2006). For the whole grain samples, 25 whole seeds were positioned in the perforations of the cooker, placed in an aluminium pan with 1500 mL of deionised water and it was cooked. For dehulled samples test, 25 split seeds were used for the analysis because most of the bambara groundnut seeds

after dehulling, micronisation and drying resulted in split seeds. The cooking times of the unmicronised and micronised bambara groundnut seeds were evaluated when 80% of the pins (49.7 g /pin) fell through the softened seeds.

Water absorption during soaking

Water absorption of bambara groundnut seeds during soaking was determined using a rapid screening procedure described by Waniska & Myers (2006) with modification. Approximately 10 g of unmicronised and micronised bambara groundnut seeds (whole and dehulled) were placed in 500 mL Erlenmeyer flasks containing 60 mL of deionised water. The flasks were placed in an incubator at 25 ºC for 1, 6, 12, 18, and 24 h. After soaking, the excess water was drained using a metal sieve (2.5 mm) aperture and the seeds were blotted dry to remove excess water and weighed. The gain in weight was expressed as g water absorbed kg^{-1} bambara groundnut seeds.

Water absorption during cooking

The amount of water absorbed by bambara groundnut seeds during cooking was determined using a rapid screening procedure as described Waniska & Myers (2006) with modification. Approximately 5 g of unmicronised and micronised pre-soaked bambara groundnut seeds (dehulled and whole samples) were placed in a plastic bags filled with 60 mL of deionised water. The bags were held by rods and placed in a large cooking container of boiling distilled water (95 ºC) for 30, 60, 90, 120 and 150 min. After cooking, the broth was drained and the seeds were blotted dry to remove excess water and weighed. The gain in weight was expressed as g water absorbed kg^{-1}bambara groundnut seeds.

Light microscopy (LM)

Small (3 × 3 × 5 mm) pieces from cotyledons of unmicronised and micronised bambara groundnut seeds (whole and dehulled) were fixed for 1 h in 2.5% glutaraldehyde in 0.075 M phosphate buffer, pH 7.4 at room temperature. It was then rinsed three times, 10 min each in 0.075 M phosphate buffer and dehydrated in ethanol (30%, 50%, 70%, 90% and 100%). The dehydrated samples were percolated with 50% LR white resin and ethanol for 2 h and infiltrated with pure LR white resin for 4 h. The resin was polymerised at 60 ºC for 24 h. Ultra-thin sections (1 µm) were cut with a diamond knife, using a Reichert-Jung/ultracut E ultramicrotome (Vienna, Austria). The sections were then transferred onto droplets of water on a specimen slide and stained with Toluidine blue for cell wall material. The slide was then viewed using a Nikon optiphot transmitted light microscope (Tokyo, Japan) fitted with

appropriate illumination sources and filters and pictures were captured with a Nikon digital camera DXM1200 (Tokyo, Japan).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of unmicronised and micronised bambara groundnut seeds (whole and dehulled) was done by first freezing the sample using liquid nitrogen and freeze fracture a small fraction. The fractions were mounted on aluminium stubs with the aid of double-sided carbon tape, followed by coating with the gold of about 20 nm in thickness. The coated samples were viewed and photographed using a JEOL JSM-840 scanning electron microscope (Tokyo, Japan) at an accelerating voltage of 5.0 kV.

Soluble pectin

Soluble pectin was determined in unmicronised and micronised bambara groundnut seeds (whole and dehulled) as described by Ndungu et al. (2012) with modification. The removal of soluble sugar was first done on the 5 g of ground flours from pre-soaked unmicronised and pre-soaked micronised bambara groundnut seed samples with ethanol (95%). The pellet (alcohol-insoluble solids) was then vacuum dried at ambient temperature (25 ºC) and stored in a desiccator. The alcohol-insoluble solids (AIS) sample was extracted with cold water and hot water. The about 1 g portion of the alcohol-insoluble solids (AIS) pellet sample was extracted three times with 10 mL of distilled water for 10 min. The extract was considered and used to determine the cold water-soluble pectin (CWSP) and hot water-soluble pectin (HWSP). Pectin content of each fraction was expressed as galacturonic acid as determined by the meta hydroxydiphenyl method of Blumenkrantz & Asboe-Hansen (1973) using galacturonic acid as a standard.

Statistical analyses

The experiment was repeated three times and data generated were analysed using Statistica version 11 (StatSoftInc, Tulsa, USA). One-way analysis of variance (ANOVA) at $p \leq 0.05$ was performed on measured values such as moisture content, water absorption and cooking time of the whole and dehulled bambara groundnut seeds sample separately. This was because whole and dehulled bambara groundnut seeds after the treatment resulted in different forms (whole and split seeds respectively). Multi-factor analysis of variance (MANOVA) at $p \leq 0.05$ was performed on soluble pectin values of the resulting flours from a whole and dehulled bambara groundnut seeds samples (similar form). The least significant difference (LSD) test at $p \leq 0.05$ was used to separate significant means. Independent variables were

micronisation time (5, 10 and 15 min) and dehulling treatment. Dependent variables were the measured values.

4.1.3 Results and discussion

4.1.3.1 Moisture content

The pre-soaked bambara groundnut seeds attained 53% moisture level after soaking in water at 25 ºC for 24 h (Table 4.1). This 53% moisture level achieved by the pre-soaked unmicronised bambara groundnut seeds was higher than moisture level of about 40% achieved by pre-conditioning of other legumes grains such as cowpea (Kayitesi et al., 2012; Mwangwela et al., 2006) and lentils (Scanlon et al., 2005). The percentage moisture content of pre-soaked unmicronised and pre-soaked micronised bambara groundnut seeds (whole and dehulled) at 130 ºC at 5, 10 and 15 min and after drying (50 ºC) for 12 h using hot air drier are shown in Table 4.1. An increase in micronisation time (5, 10 and 15 min) significantly ($p< 0.01$) reduced the moisture content of pre-soaked whole bambara groundnut seeds (Table 4.1). The moisture contents of pre-soaked micronised whole bambara groundnut seeds at 5, 10 and 15 min significantly were 43, 34 and 23%. The percentage moisture of pre-soaked unmicronised dehulled bambara groundnut was about 50% and the reduction in the percentage moisture content of micronised dehulled bambara groundnut seed samples at 5, 10 and 15 min were 41, 33 and 21% (Table 4.1). The final moisture content of the bambara groundnut seed samples after the drying (50 ºC) for 12 h were between 7 to 12%.

Similar reduction in moisture between 10- 17% have been reported in different pre-conditioned micronised legume such as cowpea (Kayitesi et al., 2012; Ndungu et al., 2012; Mwangwela et al., 2006), lentil (Arntfield et al., 2001) and other legumes (Fasina et al., 2001). Arntfield et al. (2001) reported that the reduction in the moisture of pre-conditioned (33%) micronised (138 & 170 ºC) lentil could be due to the increase in internal temperature of seeds leading to the water evaporation from collapsed small capillaries within the seed during micronisation.

4.1.3.2 Cooking time

The effect of micronisation (5, 10 and 15 min) on the cooking time of pre-soaked whole and dehulled bambara groundnut seeds is presented in Table 4.2. The cooking time was indicated time taken by 80% of pins to fell through the softened seeds. The cooking time of 109 min

was recorded for the pre-soaked whole bambara groundnut samples. A significant ($p<0.001$) reduction in the cooking time of 49, 54 and 62% were observed in pre-soaked whole bambara groundnut samples micronised at 5, 10 and 15 min respectively. The cooking time of 41min was observed for pre-soaked unmicronised dehulled bambara groundnut sample and in the same way, the cooking time of pre-soaked dehulled bambara groundnut samples micronised at 5, 10 and 15 min were reduced by 7, 10 and 22%. The reduction in the cooking time of pre-soaked micronised bambara groundnut seeds could be attributed to the changes in the hydration of the seeds during soaking and cooking. Different authors have reported that micronisation (130-170 ºC) reduced the cooking time of pre-conditioned (30 – 40% moisture) whole grain legume seeds such as cowpea of 57- 97 min by 28 – 49% (Kayitesi et al., 2012; Mwangwela et al., 2006), lentil of 30 min by 50% (Arntfield et al., 2001; Cenkowski & Sosulski, 1997) and split pea of 20 min by 33% (Cenkowski & Sosulski, 1998).

4.1.3.3 **Water absorption during soaking**

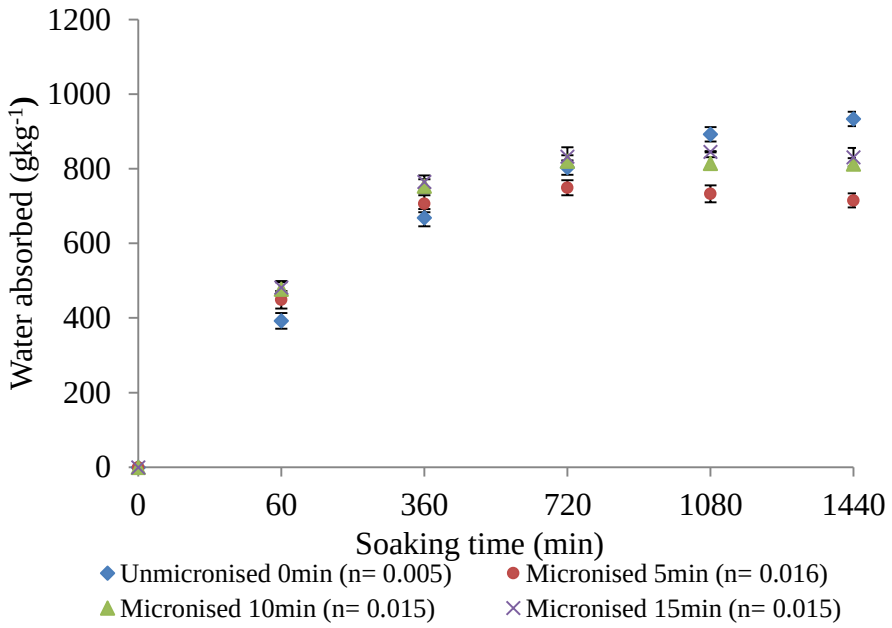
The effects of micronisation of pre-soaked whole and dehulled bambara groundnut seeds samples on their water absorption during 24 h of soaking (25 ºC) are presented in Figure 4.3. The amount of water uptakes in the unmicronised and micronised whole bambara groundnut samples significantly ($p<0.01$) increase during the 24 h of soaking (25 ºC) and thereafter plateau (Fig 4.3A). The water absorbed by the micronised whole bambara groundnut samples was higher than the unmicronised whole sample. The plateau observed after 12 h soaking of micronised samples (5, 10 and 15 min) could be an indication of saturation of water in the hydrophilic biomolecules such as starch, protein and pectin. The data of the non-linear curves of water absorption for the bambara groundnut seed samples (Figure 4.3) were fitted into an exponential regression model of $Y = Y_o + Ae^{-t/n}$ (Exponential decay first order) using OriginPro software to determine the rate of water absorption of the samples. Where: Y= % amount of water absorbed (amount of water absorbed at time (t)/ initial amount of water absorbed), Y_o = Estimated initial water absorbed at a given time, A= Amplitude of the curve, n are the slope/ Rate (g kg^{-1}/ min) and t = time (min). The data fit to the equation with the R^2-values of at least 0.92 for all the samples. The rate of water absorption at 12 h of soaking (25 ºC) of unmicronised whole bambara groundnut seeds is 0.012 (g kg^{-1}/min). The rates of water absorption in pre-soaked micronised whole bambara groundnut seeds at 5, 10 and 15 min were 0.015, 0.015 and 0.016 (g kg^{-1}/min) respectively.

Table 4.1 Effects of micronisation on some physico-chemical characteristics of whole and dehulled bambara groundnut seeds

	Reference	Control	Micronisation time (min)		
Characteristics	Raw	0	5	10	15
Whole samples					
Moisture content before drying (%)	8.0 (0.1)	$53.0^{d1}(1.3)^{2}$	43.2^{c} (1.0)	34.0^{b} (0.8)	23.0^{a} (3.0)
Moisture content after 12 h drying (%)	ND	11.0^{b} (0.2)	12.1^{c} (0.1)	11.0^{b} (0.3)	8.3^{a} (0.3)
Cooking time after 12 h drying (min)	162.0 (2.0)	109.0^{d} (2.0)	83.0^{c} (3.0)	75.0^{b} (1.0)	62.3^{a} (2.0)
Reduction in cooking time (%)	ND	33	49	54	62
Dehulled samples					
Moisture content before drying (%)	ND	50.2^{d} (0.3)	41.1^{c} (1.1)	33.0^{b} (0.1)	21.4^{a} (0.1)
Moisture content after 12 h drying (Seeds) (%)	ND	10.0^{b} (0.3)	10.0^{b} (3.0)	11.1^{b} (0.2)	7.2^{a} (0.3)
Cooking time after 12 h drying (min)	ND	41.0^{c} (1.0)	38.3^{b} (1.0)	37.0^{b} (1.0)	32.0^{a} (1.0)
Reduction in cooking time (%)	ND	-	7	10	22

1: Means with different superscripts in rows differ significantly ($p \leq 0.05$) and differ for whole and dehulled samples
2: Standard deviation in parenthesis
3: Condition of micronisation of pre-soaked bambara groundnut seeds: 53% moisture, 130 ºC surface temperature.ND: Not determined; Samples:Raw whole seeds (reference material) 0 min (unmicronised whole seeds); micronised whole seeds (5, 10 and 15 min) Samples: 0min (unmicronised dehulled seeds); micronised dehulled seeds (5, 10 and 15 min). Control (pre-soaked and unmicronised samples); treatments (pre-soaked and micronised samples at 5,10 & 15 min).

(A)



(B)

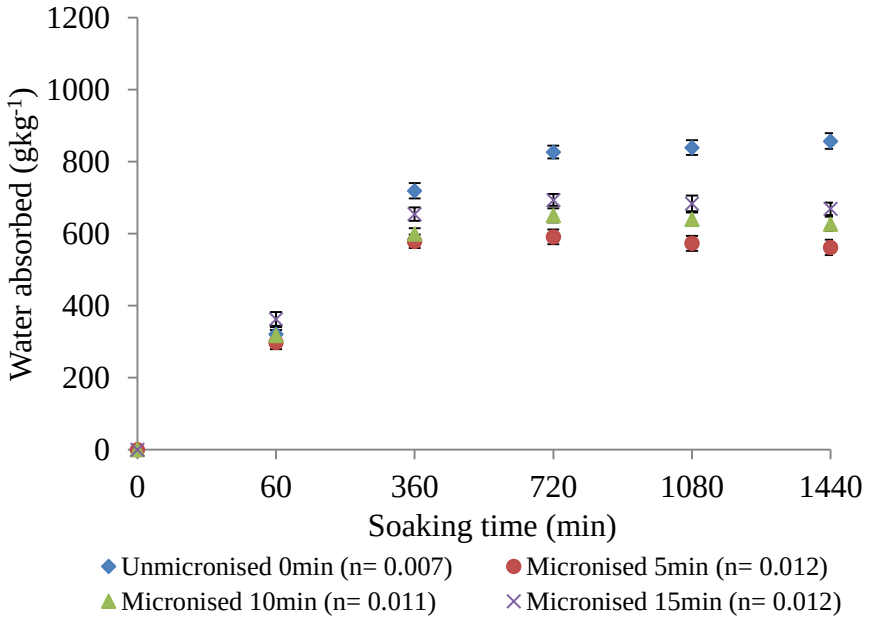


Figure 4.3 Effects of micronisation on water absorption of whole (A) and dehulled (B) bambara groundnut seeds during 24 h of soaking at ambient temperature (25 ºC)

Condition of micronisation of pre-soaked bambara groundnut seeds: 53% moisture, 130 ºC surface temperature for 5, 10 and 15 min. Control (pre-soaked and unmicronised samples); treatments (pre-soaked and micronised samples at 5,10 & 15 min). n-values (Slopes on the Exponential first order): Rate of water absorbed (g kg^{-1}/min).

The rate of water absorption of the micronised whole samples was higher than the unmicronised samples, while the rate of water absorption was similar with the micronisation.

The water absorption of unmicronised and micronised dehulled bambara sample significantly ($p<0.01$) increase during the soaking and thereafter plateau. Micronisation significantly ($p<0.01$) increase the amount of water absorbed during 24 h of soaking (25 ºC) of dehulled bambara groundnut seed samples and thereafter plateau in the first 6 h. The amount of water absorbed by micronised dehulled bambara groundnut seeds was slightly higher than the unmicronised samples in the first hour of the soaking (Fig 4.3B). Similarly, at 12 h of soaking (25 ºC) the rate of water absorption of dehulled bambara groundnut samples micronised at 5, 10 and 15 min (0.012, 0.011 and 0.012 g kg^{-1}/min respectively) were higher than the unmicronised samples (0.010 g kg^{-1}/min).

The improvement observed in the amount of water absorbed and the rate of water absorption in both whole and dehulled bambara groundnut during soaking due to the micronisation could partly be responsible for the reduction in the cooking time (Table 4.1). This may be attributed to the change caused by micronisation on the microstructure of the pre-soaked bambara groundnut seeds. Several authors have reported an improvement in hydration of pre-conditioned micronised legumes such as whole pinto (Abdul-Kadir et al., 1990), lentil (Cenkowski & Sosulski, 1997), split peas (Cenkowski & Sosulski, 1998), cowpea (Mwangwela et al., 2006) and other legumes (Fasina et al., 2001) during soaking. Mwangwela et al. (2006) attributed the improvement in water uptakes by pre-conditioned (41% moisture) micronised cowpea (153 ºC) in the first hour of soaking (22 ºC) to the physical fissures on seed coats and cotyledon and the creation of the cavity within cotyledon due to the escaping vapour during micronisation.

Most research reported above have shown water absorption of whole grain rather than dehulled legume grain. In this paper, water absorption of dehulled bambara groundnut seeds are reported. Seed coat in most legume seeds act as the first barrier to water passage into the cotyledon (Fasina et al., 1999) and its removal in different varieties of cowpea seeds was found to improve their rate of water uptakes during soaking (Penicela, 2011).The absence of seed coat could contribute to water absorption improvement in dehulled bambara groundnut samples. Also, the seeds split

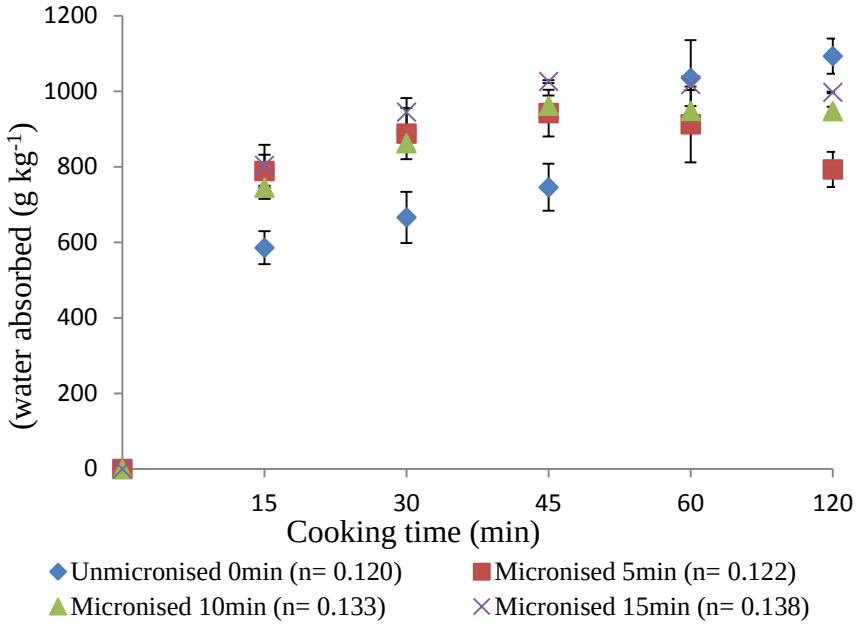
occurrence (two separate halves) and the increase in seeds surface area due to the dehulling could contribute to the rapid increase in water uptakes through their cotyledons.

4.1.3.4 **Water absorption during cooking**

Figure 4.3 shows the effect of micronisation of pre-soaked bambara groundnut (whole and dehulled) samples on their water absorption during 2 h of cooking (95 ºC). The water uptakes in the unmicronised whole bambara groundnut sample significantly ($p<0.01$) increased during the 2 h of cooking (95 ºC). Likewise, micronisation significantly ($p< 0.01$) increased the amount of water absorption during 2 h cooking (95 ºC) of whole bambara groundnut seeds samples in the first 45 min of cooking thereafter plateau (Fig. 4.4A).

The amount of water absorbed in whole bambara groundnut seeds samples with micronisation is higher than the unmicronised whole sample during cooking (95 ºC). The rate of water absorption of unmicronised whole bambara groundnut seeds at 30 min of cooking is 0.120 (g kg^{-1}/min). The rate of water absorption of whole bambara groundnut seeds micronised at 5, 10 and 15 min were 0.122, 0.133 and 0.138 (g kg^{-1}/min) respectively. The rate of the water absorption in micronised whole bambara groundnut samples was higher than the unmicronised samples. Similarly, the increase observed in the amount of water absorbed and the rate of water absorption of whole bambara groundnut due to micronisation during cooking. The rapid increase in the water uptakes of micronised bambara groundnut seeds could facilitate the gelatinisation of starch, denaturation of protein and solubilisation of pectin during the cooking. This may be responsible for the reduction in the cooking time (Table 4.1). Which may be attributed to the changes caused by micronisation on the microstructure and biomolecules such as starch, protein and pectin of the pre-soaked bambara groundnut seeds. The water absorption significantly ($p<0.01$) increase in the first 45 min of cooking (95 ºC) and thereafter plateau in dehulled samples (Fig. 4.4B). In addition, micronisation caused a slight increase on the amounts of water absorbed during the 2 h of cooking (95 ºC) of unmicronised and micronised dehulled samples. There was an increase in the amount of water absorbed with micronisation (5, 10 and 15 min) of dehulled bambara groundnut seeds compared to unmicronised. This was noticed at the first 30 min of cooking which thereafter plateau (Fig. 4.4B).

(A)



(B)

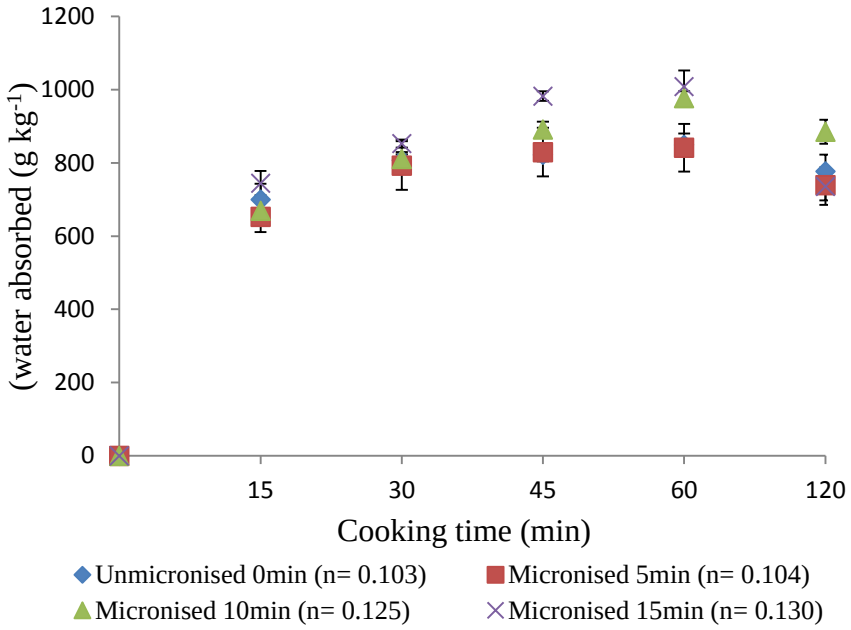


Figure 4.4 Effects of micronisation on water absorption of whole (A) and dehulled (B) bambara seeds during 2 h of cooking at boiling temperature (95 ºC)

Condition of micronisation of pre-soaked bambara groundnut seeds: 53% moisture, 130 ºC surface temperature for 5, 10 and 15 min. Control (pre-soaked and unmicronised samples); treatments (pre-soaked and micronised samples at 5,10 & 15 min). n-values (Slopes on the Exponential first order): Rate of water absorbed (g kg^{-1}/min).

The water absorption of the dehulled samples (0, 5, 10 and 15min) significantly ($p<0.01$) decrease at 120 min of cooking (95 ºC). The rate of water absorption in dehulled unmicronised

bambara groundnut sample at 30 min of cooking (95 ºC) is 0.103 (g kg^{-1}/min). The rate of water absorption in dehulled bambara groundnut seeds micronised for 5, 10 and 15 min during 2 h cooking (95 ºC) were 0.104, 0.125 and 0.130 (g kg^{-1}/min) respectively. Similarly, the rate of water absorption observed during cooking of micronised dehulled bambara groundnut samples was higher compared to the unmicronised sample.

The increase in water absorption during cooking have been reported in whole pre-conditioned micronised cowpea (41% moisture, 153 ºC) (Mwangwela et al., 2006) and pea (10% moisture, 180 ºC) (Andrejko et al., 2008), due to structural changes such as development of fissures in micronised pre-conditioned seeds and changes in biomolecules such as starch (Mwangwela et al., 2006). The authors reported that during cooking for 90 min, air spaces (capillary) and intercellular spaces in pre-conditioned micronised cowpea seeds could improve seeds hydration. Dehulling was reported not to actually be important in the water absorption of seeds during cooking (Penicela, 2011). The decrease in water absorption of dehulled samples during cooking could be due distruption in the integrity of the cotyledons cooking for 120 min and this resulted in the leaching of solids into the boiling water. This therefore, contributed to the under estimation of the water absorption in the dehulled samples particularly at 120 min of cooking. In order to understand the improvement in water uptakes and reduction of cooking time of micronised pre-soaked bambara groundnut, the analyses investigating the changes in their structural and molecular components were determined.

4.1.3.5 **Light Microscopy (LM)**

The micrographs of pre-soaked (53% moisture) unmicronised and micronised whole and dehulled bambara groundnut seeds cotyledon using toluidene stained for cell wall material and middle lamella under plane light microscopy (LM) are shown in Figure 4.5. The micrographs (LM) show the starch granules of pre-soaked unmicronised bambara groundnut seeds samples (whole and dehulled) were round or oval in shapes. The parenchyma cells in the cotyledon of the unmicronised samples were also round in shapes and compactly bounded within a thin cell wall.

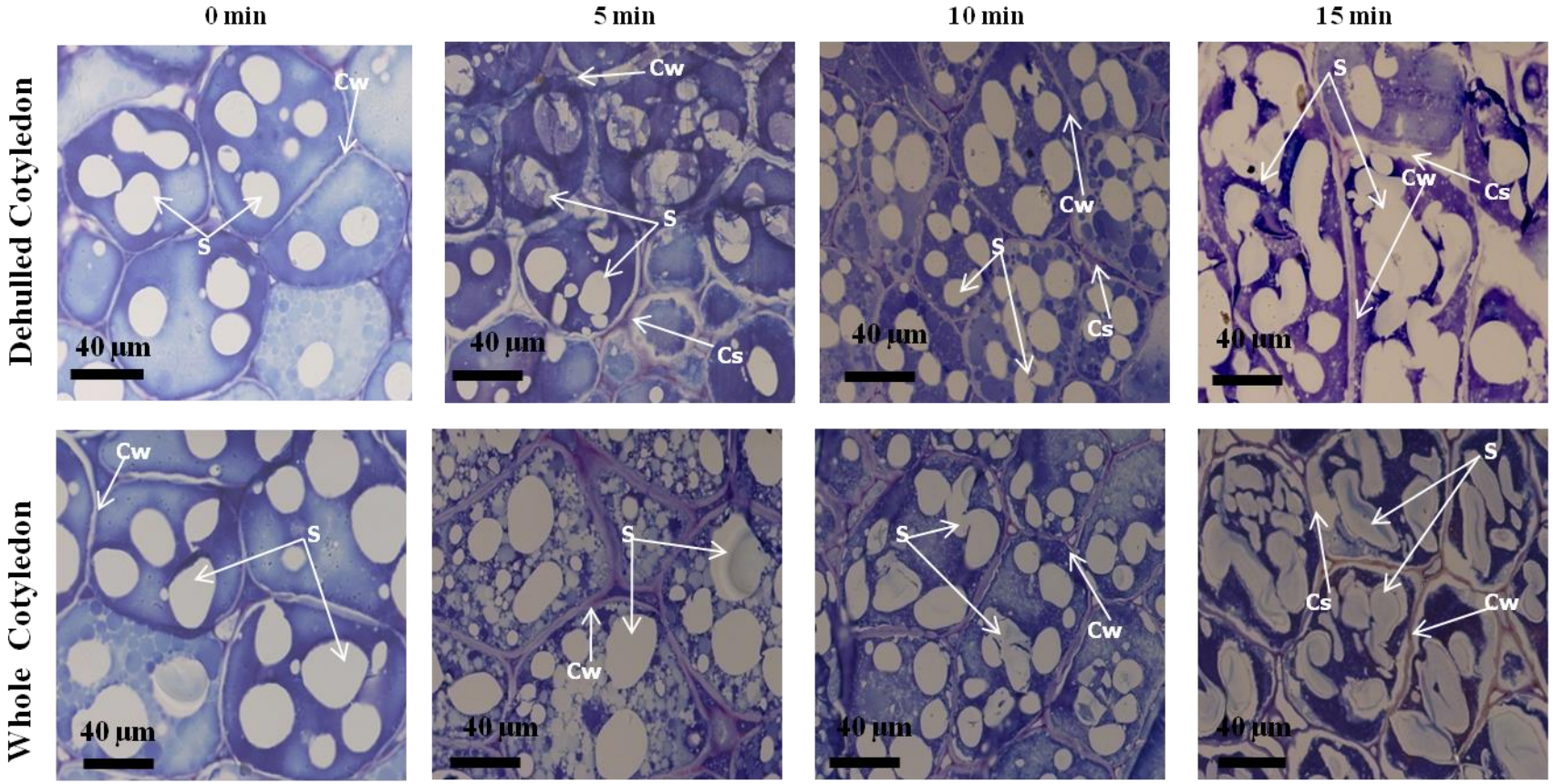


Figure 4.5Light micrographs of toluidene stained unmicronised and micronised bambara groundnut seeds cotyledon (Whole grain and Dehulled).

Condition of micronisation of pre-soaked bambara groundnut seeds: 53% moisture,130 ºC surface temperature for 5, 10 and 15 min. Control (pre-soaked and unmicronised samples); treatments (pre-soaked and micronised samples at 5,10 & 15 min). Cw, cell wall: Cs, Cell separation: S, Starch granules.

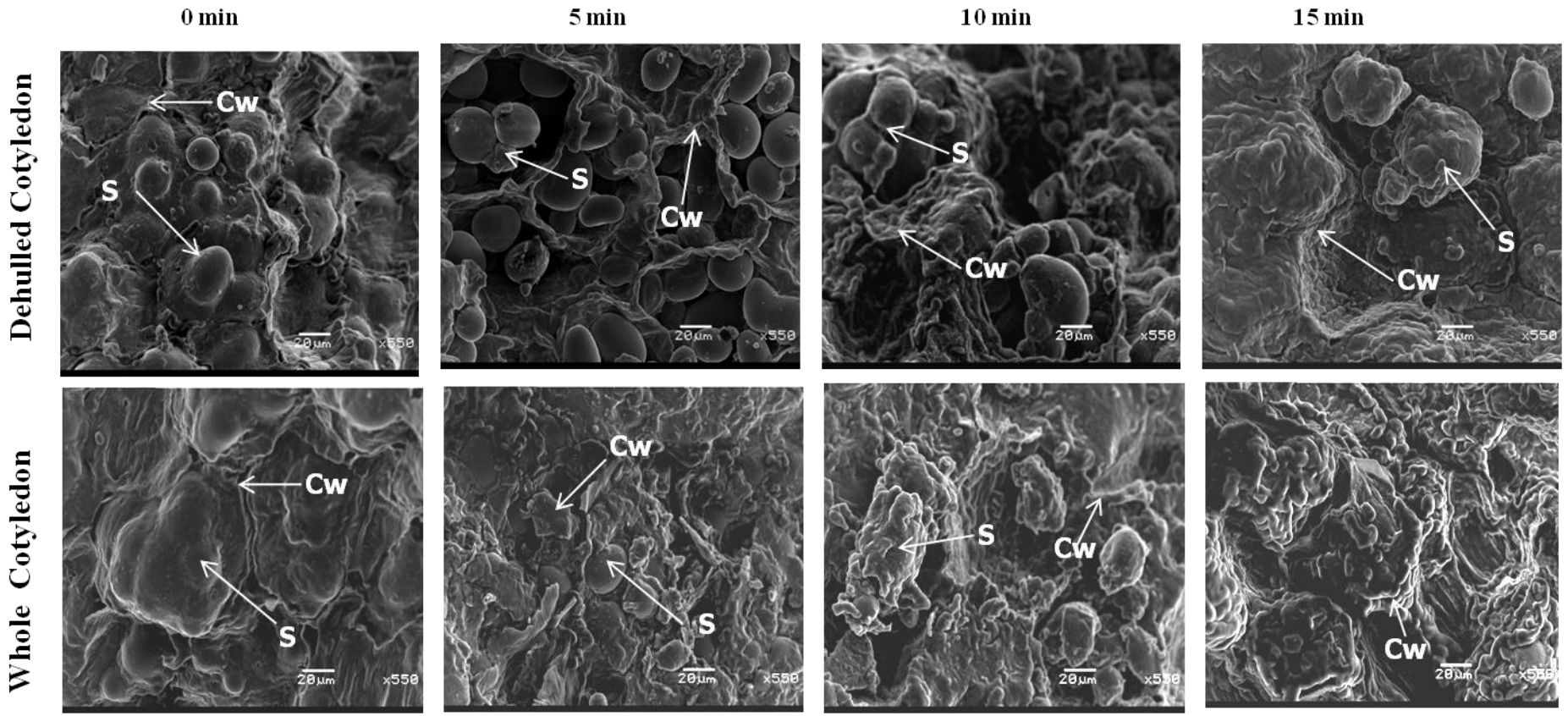


Figure 4.6 Scanning electron micrographs of unmicronised and micronised bambara groundnut seeds cotyledon (Whole grain and Dehulled).

Condition of micronisation of pre-soaked bambara groundnut seeds: 53% moisture, 130 ºC surface temperature for 5, 10 and 15 min. Control (pre-soaked and unmicronised samples); treatments (pre-soaked and micronised samples at 5,10 & 15 min). Cw, cell wall: S, Starch granules.

As observed in the micrographs of pre-soaked micronised bambara groundnut seeds (whole and dehulled samples) (Fig. 4.5), micronisation altered the shapes of starch granules as an indication of possible starch granules disruption. Also, changes were observed in the shapes of the parenchyma cells of the pre-soaked micronised bambara groundnut seeds with micronisation. The thickening of the cell walls (which suggest possible disintegration of the middle lamella) and cell separation were also observed in pre-soaked micronised bambara groundnut seeds.

4.1.3.1 **Scanning electron microscopy (SEM)**

Additionally, the micrographs of pre-soaked (53% moisture) unmicronised and micronised whole and dehulled bambara groundnut seeds cotyledon using scanning electron microscopy (SEM) were in Figure 4.6. Cell components such as starch granules in cotyledon of pre-soaked unmicronised bambara groundnut (whole and dehulled) samples were found to be round or oval with a smooth surface. The parenchyma cells of pre-soaked unmicronised bambara groundnut had intact cell walls. It was observed that micronisation changes shapes and structurally disrupted the cell components such as starch granules, the disrupted starch granules were coated with proteinaceous and/or unattached particles of other cell components. The cell walls were collapsed and an observation of cell wall reminants was also made which could suggest possible damage of protein matrix due to the micronisation.

The change observed in the structure of cotyledons of micronised bambara groundnut (Fig 4.5 and Fig 4.6) suggest it could facilitate the water absorption during soaking and cooking. As a result of the rapid increase in water uptakes especially during cooking could then partly be responsible for the reduction in cooking time.

Cell separation along the middle lamella and the presence of cell wall remnants in the cotyledon have been reported in micronised cowpea (41% moisture, 153 ºC) (Mwangwela et al., 2006) and lentils (33% moisture, 138 ºC) (Arntfield et al., 2001). Cenkowski & sosulski (1998) reported damaged starch granules coated with proteinaceous in micronised split pea. The structural changes on seed coats and cotyledon as a result of micronisation have been reported to facilitate higher water uptakes in pre-conditioned micronised whole legume seeds during cooking (Mwangwela et al., 2006).

4.1.3.2 **Soluble pectin**

Pectin solubilisation is one of the most important molecular changes among others necessary for cell separation and softening of legumes seeds (Ndungu et al., 2012). The thickening of cell walls and cell separation as observed (Fig 4.4) could indicate the possibility of pectin solubilisation in the pre-soaked micronised bambara groundnut seed samples. Table 4.2 shows the effect of micronisation on the soluble pectin (cold water pectin solubility) content of pre-soaked bambara groundnut samples. The cold water soluble pectin content in pre-soaked unmicronised whole bambara groundnut sample is 98.1 mg/100g. The cold water pectin solubility content (CWPS) of whole bambara groundnut sample significantly ($p< 0.001$) increased with micronisation by 123.8, 127.2 and 131.0 mg/100g for 5, 10 and 15 min respectively. Similarly, the cold water soluble pectin content in pre-soaked unmicronised dehulled bambara groundnut sample is 89.8 mg/100g and this increased with micronisation to 90.4, 108.3 and 118.5 mg/100g for 5, 10 and 15 min respectively.

In addition, Table 4.2 also shows the effect of micronisation on the hot water pectin solubility (HWPS) content of the pre-soaked bambara groundnut (whole and dehulled) samples. The hot water soluble pectin content of pre-soaked unmicronised whole bambara groundnut sample is 128.6 mg/100g. The hot water soluble pectin content significantly ($p< 0.001$) increased (whole bambara groundnut samples) with micronisation to 184.4, 188.0 and 214.4 mg/100g for 5, 10 and 15 min respectively. The hot water soluble pectin content of pre-soaked unmicronised dehulled bambara groundnut sample is 88.74 mg/100 g and this significantly ($p< 0.001$) increased likewise with micronisation by 131.0, 139.4 and 153.0 mg/100 g for 5, 10 and 15 min respectively.

The higher total soluble pectin content of pre-soaked micronised bambara groundnut than unmicronised samples (Table 4.2), shows that micronisation of pre-soaked micronised bambara groundnut could increase the formation of water soluble pectin during soaking and cooking. Also, the higher total soluble pectin content (total soluble pectin) in the whole bambara groundnut samples than the dehulled samples, could be due to presence of more non starch polysaccharides in the whole seed than the cotyledon of the seeds without the seed coat (Wang et al., 2009). The higher content of hot water soluble pectin (HWPS) than cold water soluble pectin

(CWPS) showed there could be an increase in the pectin solubility when extracted with the hot water. The increase in pectin solubility has been attributed to the degradation of pectin via the β-elimination reaction, leading to dissociation of hydrogen bond linkages in pectin polymers (Ndungu et al., 2012). The increase in soluble pectin content (Table 4.2) with micronisation could be related to the structural changes in cell wall material (Figures 4.4 & 4.5). This could also be responsible for the rapid water uptakes (Figures 4.3 & 4.4) and reduction in the cooking of the pre-soaked micronised bambara groundnut seeds (Table 4.1).

Table 4.2 Effects of micronisation on pectin solubility of whole and dehulled bambara seeds

	Micronisation time (min)			
Cold water pectin solubility (mg/100g)	0	5	10	15
Whole samples	98.1^{ab} (2.0)	123.8^{de} (3.0)	127.2^{de} (6.2)	131.0^{e} (1.3)
dehulled samples	89.8^{a} (6.1)	90.4^{a} (5.6)	108.3^{bc} (4.0)	118.5^{dc} (9.0)
Hot water pectin solubility (mg/100g)				
Whole samples	128.6^{b} (8.4)	184.4^{d} (8.0)	188.0^{d} (1.3)	214.4^{e} (4.3)
dehulled samples	88.7^{a} (4.6)	131.0^{b} (1.0)	139.4^{bc} (9.7)	153.0^{c} (7.3)
Total soluble pectin (mg/100g)				
Whole samples	226.7	308.2	315.2	345.4
dehulled samples	178.5	221.4	247.7	271.5

1: Means with different superscripts in rows differ significantly ($p \leq 0.05$)
2: Standard deviation in parenthesis
3:Condition of micronisation of pre-soaked bambara groundnut seeds: 53% moisture,130 ºC surface temperature for 5, 10 and 15 min. Control (pre-soaked and unmicronised samples); treatments (pre-soaked and micronised samples at 5,10 & 15 min).

Similarly, the increase in soluble pectin content has been reported in pre-conditioned micronised cowpea (Ndungu et al., 2012) and lentil (Arntfield et al., 2001) as compared to the unmicronised samples. The pectin solubility has been related with the disintegration of middle lamella and causing cell separation during cooking of micronised whole seeds (Mwangwela et al., 2006). Micronisation of pre-conditioned whole legume seeds such as cowpea (Mwangwela et al., 2006) and lentil (Arntfield et al., 2001) have also been reported to cause starch gelatinisation and

protein denaturation. Ndungu et al. (2012) related the increase in pectin solubilisation to the reduction in cooking time of pre-conditioned and micronised cowpea, and an increase in the degree of starch gelatinisation could also be associated with rapid water absorption and shorter cooking times of micronised legume seeds (Kayitesi et al., 2012).

The reduction in the cooking time of pre-soaked (53%) micronised whole bambara groundnut seeds (bigger seeds) (Table 4.1), especially at 15 min (62%) was higher than earlier reported by other authors on micronised pre-conditioned (40% moisture) whole grain legumes such as cowpea (Kayitesi et al., 2012; Mwangwela et al., 2006) with smaller seed size. This suggests at this moisture (53%) due to micronisation, the energy absorption and molecular vibration of moisture and other biomolecules such as protein starch and pectin could increase. Then resulted in an increase the heat generation and escaping of water vapour in the cotyledon. Therefore, enhancing more structural changes such as fissures on seed coat and cotyledon and molecular changes such as starch pre-gelatinisation, protein denaturation and pectin solubilisation in pre-soaked micronised bambara groundnut seeds than reported pre-conditioned micronised legume seeds (Kayitesi et al., 2012; Mwangwela et al., 2006; Arntfield et al., 2001; Cenkowski & Sosulski, 1998; Cenkowski & Sosulski, 1997). This possibly facilitated the transfer of water across the cell wall and cotyledons of micronised seeds samples during cooking thereby enabling the seeds to soften more quickly. The cooking time of whole and dehulled bambara groundnut samples was not comparable because of their different forms. With micronisation of dehulled bambara groundnut (split of the seed cotyledon into two halves) at about 50% moisture, could possibly result in an increase in the fissures on the cotyledon and molecular changes such as starch pre-gelatinisation, protein denaturation and pectin solubilisation. Therefore, this could facilitate the water uptakes of the dehulled micronised bambara groundnut samples and may be responsible for the reduction in cooking time.

4.1.4 Conclusions

Bambara groundnut seeds pre-soaked to 53% and micronised (5, 10 and 15 min) causes a rapid increase of about 24 to 32% in water absorption to pre-soaked unmicronised (701 g kg^{-1}) during cooking (30 min). The increases in their total water soluble pectin (36 - 52%) to the pre-soaked unmicronised (226 mg/100g) and changes in microstructure of cotyledons associate with the rapid water uptake. This is responsible for the reduction in their cooking time (49- 62%) to pre-

soaked unmicronised at 109 min. Dehulling play more significant role in reduction of cooking time (41 min) of the bambara groundnut seeds . Therefore, bambara groundnut seeds could be process to a short time cooking legume for consumers convinient, thus improving its consumption and utilisation.

4.1.5 References

Abdul-Kadir, R., Bargman, T.J., & Rupnow, J.H., 2006. Effect of infrared heat processing on rehydration rate and cooking of *Phaseolus vulgaris (var. Pinto).Journal of Food Science*, 55, 1472-1473.

Abu, J. O., & Minnaar, A., 2009. Gamma irradiation of cowpea *(Vigna unguiculata L. Walp)* seeds: effect on colour, cooking quality and pasting characteristics. *International Journal of Food science & Technology*, 44, 2335-2341.

Ajibola, O., Aviara, N., & Ajetumobi, O., 2003.Sorption equilibrium and thermodynamic properties of cowpea *(Vigna unguiculata).Journal of Food Engineering*, 58, 317-324.

Alajaji, S. A., & El-Adawy, T. A., 2006. Nutritional composition of chickpea *(Cicer arietinum L.)* as affected by microwave cooking and other traditional cooking methods.*Journal of Food Composition and Analysis*, 19, 806-812.

Andrejko, D., Rydzak, L., Slaska-Grzywna, B., Goÿdziewska, M., & Kobus, Z., 2008. Influence of preliminary thermal processing applying infra-red radiation on pea seeds cooking process. *International Agrophysics*, 22, 17-20.

Annan, N., Plahar, W., & Swetman, T., 2002. Effect of kawe treatment on the water absorption and cooking characteristics of bambara groundnut varieties. *Food Research Institute (FRI)*, Accra, Ghana.15 pp.

Arntfield, S. D., Scanlon, M. G., Malcolmson, L. J., Watts, B., Ryland, D., & Savoie, V., 1997.Effect of tempering and end moisture content on the quality of micronised lentils.*Food Research International*, 30, 371-380.

Arntfield, S.D., Scanlon, M.G., Malcolmson, L.J., Watts, B.M., Cenkowski, S., Ryland, D., & Savoie, V., 2001. Reduction in lentil cooking time using micronisation: Comparison of two micronisation temperatures. *Journal of Food Science*,66, 500-505.

Azam-Ali, S., Sesay, A., Karikari, S., Massawe, F., Aguilar-Manjarrez, J., Bannayan, M., & Hampson, K., 2001. Assessing the potential of an underutilized crop–a case study using bambara groundnut. *Experimental Agriculture*, 37, 433-472.

Blumenkrantz, N., & Asboe-Hansen, G., 1973. New method for quantitative determination of uronic acids.*Analytical Biochemistry*, 54, 484-489.

Carman K., 1996. Some physical properties of lentil seeds.*Journal of Agricultural Engineering Research*, 63(2), 87-92.

Cenkowski, S., & Sosulski, F., 1998. Cooking characteristics of split peas treated with infrared heat. *Transactions of the ASAE*, 41, 715-720.

Cenkowski, S., & Sosulski, F.W., 1997. Physical and cooking properties of micronized lentils. *Journal of Food Process Engineering*, 20, 249-264.

Fasina, O., Tyler, R., Pickard, M., & Zheng, G., 1999. Infrared heating of hulless and pearled barley. *Journal of Food Processing and Preservation*, 23, 135-151.

Fasina, O., Tyler, B., Pickard, M., Zheng, G.H., & Wang, N., 2001. Effect of infrared heating on the properties of legume seeds. *International Journal of Food Science & Technology*, 36, 79-90.

Kabas O., et al., 2007. Some physical and nutritional properties of cowpea seed *(Vignasinensis L.). Journal of Food Engineering*, 79(4), 1405-1409.

Kaptso, K. G., Njintang, Y. N., Nguemtchouin, M. M. G., Scher, J., Hounhouigan, J., & Mbofung, C. M., 2014. Physicochemical and micro-structural properties of flours, starch and proteins from two varieties of legumes: bambara groundnut *(Vigna subterranea). Journal of Food Science and Technology*, 1-10.

Kaptso, K., Njintang, Y., Komnek, A., Hounhouigan, J., Scher, J., & Mbofung, C., 2008. Physical properties and rehydration kinetics of two varieties of cowpea *(Vigna unguiculata)* and bambara groundnut *(Voandzeia subterranea)* seeds. *Journal of Food Engineering*, 86, 91-99.

Kayitesi, E., Duodu, K. G., Minnaar, A., & De Kock, H. L., 2012. Effect of micronisation of pre-conditioned cowpeas on cooking time and sensory properties of cooked cowpeas.*Journal of the Science of Food and Agriculture*, 93, 838–845.

Krishnamurthy, K., Khurana, H. K., Soojin, J., Irudayaraj, J., & Demirci, A., 2008. Infrared heating in food processing: An Overview. *Comprehensive Reviews in Food Science and Food Safety*, 7, 2-13.

Mpotokwane, S., Gaditlhatlhelwe, E., Sebaka, A., & Jideani, V., 2008. Physical properties of bambara groundnuts from Botswana. *Journal of Food Engineering*, 89, 93-98.

Mubarak, A., 2005. Nutritional composition and anti-nutritional factors of mung bean seeds *(Phaseolus aureus)* as affected by some home traditional processes. *Food Chemistry*, 89(4), 489-495.

Mune, M., Minka, S. R., Mbome, I. L., & Etoa, F., 2011. Nutritional potential of bambara bean protein concentrate. *Pakistan Journal of Nutrition*, 10, 112-119.

Mwangwela, A. M., Waniska, R. D., & Minnaar, A., 2006. Hydrothermal treatments of two cowpea *(Vigna unguiculata L. Walp)* varieties: effect of micronisation on physicochemical and structural characteristics. *Journal of the Science of Food and Agriculture*, 86, 35-45.

Mwangwela, A. M., Waniska, R. D., Mcdonough, C., & Minnaar, A., 2007a. Cowpea cooking characteristics as affected by micronisation temperature: a study of the physicochemical and functional properties of starch. *Journal of the Science of Food and Agriculture*, 87, 399-410.

Mwangwela, A. M., Waniska, R. D., & Minnaar, A., 2007. Effect of micronisation temperature (130 and 170 ºC) on functional properties of cowpea flour.*Food chemistry*, 104, 650-657.

Nti, C. A., 2009. Effects of bambara groundnut *(Vigna subterranea)* variety and processing on the quality and consumer appeal for its products. *International Journal of Food Science & Technology*, 44, 2234-2242.

Ndungu, K. E., Emmambux, M. N., & Minnaar, A., 2012. Micronisation and hot air roasting of cowpeas as pretreatments to control the development of hard-to-cook phenomenon.*Journal of the Science of Food and Agriculture*, 92, 1194-1200.

Ojimelukwe, P. C., 1999. Cooking characteristics of four cultivars of bambara groundnut seeds and starch isolate. *Journal of Food Biochemistry*, 23, 109-117.

Penicela, L., 2011. The Influence of seed coat and cotyledon structure on cooking characteristics of cowpea.MSc dissertation.(Food Science).University of Pretoria, Pretoria.

Scanlon, M. G., Cenkowski, S., Segall, K. I., & Arntfield, S. D., 2005. The physical properties of micronised lentils as a function of tempering moisture.*Biosystems Engineering*, 92, 247-254.

Subuola, F., Widodo, Y., & Kehinde, T., 2012. Processing and utilization of legumes in the tropics. *Trends in Vital Food and Control Engineering. Rijeka, Croatia: InTech*, 71.

Waniska, R., & Myers, J. R., 2006. Analysis of cooking and consumer preference traits to achieve wider impact from bean/cowpea CRSP developed bean cultivars. *Research and Training Highlights*, 39.

Wang, N., Hatcher, D., Toews, R., & Gawalko, E., 2009. Influence of cooking and dehulling on nutritional composition of several varieties of lentils *(Lens culinaris). LWT-Food Science and Technology*, 42, 842-848.

Zheng, G., Fasina, O., Sosulski, F., & Tyler, R., 1998. Nitrogen solubility of cereals and legumes subjected to micronization. *Journal of Agricultural and Food Chemistry*, 46, 4150-4157.

4.2 Effects of micronisation and dehulling of pre-soaked bambara groundnut seeds on microstructure and functionality of the resulting flours

Abstract

Functional properties of flours from pre-soaked and micronised (130 ºC) whole and dehulled bambara seeds (5, 10 and 15 min) were determined. An increase in micronisation time significantly reduced the pasting viscosity of the flours. Significant reductions in the differential scanning calorimetry endothermic peak enthalpies and loss of birefringence in the starch were found, indicating starch pre-gelatinisation when micronised. The low viscous paste of resultant flours seems to be related to protein denaturation as shown by decrease in nitrogen solubility index. Starch was embedded in a protein matrix as shown by confocal laser scanning microscopy. This denatured protein matrixes could be in part preventing starch hydration and dispersion during pasting and thus reduced viscosity. Dehulling reduced the pasting viscosity suggesting higher effect of micronisation for dehulled than whole samples. Resulting flours can be useful ingredients in protein energy-dense foods due to low viscosity.

Key words: Bambara groundnut, Pre-soaking, Micronisation, Dehulling, Resulting flour

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4.2.1 Introduction

Bambara groundnut (*Vigna subterranean*) is an indigenous underutilised legume that is widely grown in sub-Saharan African countries. It is ranked third most important grain legume after groundnut (*Arachis hypogaea L.*) and cowpea (*Vigna unguiculata*) (Afoakwa, Budu, & Merson, 2007). Bambara groundnut has a protein content of about 18-24% and carbohydrates content of about 60-63% (Eltayeb, Ali, Abou-arab, & Abu-Salem, 2011; Yusuf, Ayedun, & Sanni, 2008). Bambara groundnuts are commonly consumed as wet-cooked or roasted. However, the long cooking time of about 2h or more in boiling water is a limitation to its utilisation (Ojimelukwe, 1999). Flour from bambara groundnuts can be used in the production of stiff porridge known as okpa in Nigeria (Barimalaa, Agoha, Oboh, & Kiin-Kabari, 2005) and has shown potential as composite in bread production (Alozie, Iyam, Lawal, Udofia, & Ani, 2009). The flour from different varieties of bambara groundnut have good functional properties, e.g., foaming capacities and stability, oil and water absorption capacities, bulk density, gelation capacities and emulsifying activity (Aremu, Olaofe, & Akintayo, 2007).

The functionality of legume flour has been related to its biomolecules. Foaming, emulsification, and water and oil absorption has been closely related to function of proteins (Aremu, Olaofe, & Akintayo, 2007); viscosity and swelling characteristics related to starch (Singh, Kaur, Sandhu, & Guraya, 2004); and gelation to both starch and proteins (Prinyawiwatkul, Beuchat, Mcwatters, & Phillips, 1997). Bambara groundnut flour and its extracted protein and starch from bambara groundnut have recently been studied as a potential way to improve its utilisation in food application (Sirivongpaisal, 2008; Yusuf, Ayedun, & Sanni, 2008; Eltayeb, Ali, Abou-arab, & Abu-Salem, 2011). Functionality and nutritional quality of bambara groundnut and its flour are affected by various processing technologies, for example dehulling and hydrothermal treatments.

Dehulling pre-treatment is the removal of seed coat from pre-soaked legume seeds. It has been shown to increase the protein content, reduced antinutrients such as tannin in bambara flour (Nti, 2009) and reduced dietary fibre of flour from legume seeds such as lentils (Wang, Hatcher, Toews, & Gawalko, 2009). These changes can improve the nutritional quality of bambara groundnut flour, especially when used as infant weaning food it has good quality proteins (Nti, 2009).

Hydrothermal treatment in this research refers to micronisation of pre-conditioned legumes (Kayitesi, Duodu, Minnaar, & De Kock, 2012; Mwangwela, Waniska, & Minnaar, 2006;

Arntfield et al. 2001). Micronisation utilises electromagnetic radiation with wavelength (1.8–3.4µm) in the infrared radiation regions (Rastogi, 2012). The food material generally conditioned with moisture are exposed to the infrared radiation, the infrared wave strikes on the food surface and parts of the penetrated energy are absorbed resulting in molecular vibration by the food molecules at a frequency of 60,000- 150,000 MHz (Fasina, Tyler, Pickard, Zheng, & Wang, 2001). The molecular vibration of the biomolecules generates heat due to molecular friction; and rise in water vapour pressure in the food (Sharma, 2009). Micronisation has been found to improve the hydration (Mwangwela, Waniska, & Minnaar, 2007b: Cenkowski & Sosulski, 1998) and reduced pasting viscosities (Mwangwela, Waniska, McDonough, & Minnaar, 2007a) of resulting flours from micronised whole cowpea.

The reason for low pasting viscosity of resulting flours from micronised legume such as cowpea has not been elucidated (Mwangwela, Waniska, McDonough, & Minnaar, 2007a). Also, lower moisture condition of about 40% prior to micronisation (Kayitesi, Duodu, Minnaar, & De Kock, 2012; Arntfield et al., 2001) has been reported compared to soaking. Thus, the objective of the study was to determine the effects of micronisation and dehulling treatment of pre-soaked bambara groundnut on physicochemical, microstructure and functional properties of the resulting flours in order to improve its utilisation of their resultant flours.

4.2.2 Materials and methods

4.2.2.1 **Raw Material**

Mixed bambara groundnut seeds were obtained from Mbara produce market in Harare, Zimbabwe. The seeds were thoroughly cleaned to remove the chaff, shrivelled and broken seeds. The bambara groundnut seeds were then sorted based on the basis of seed size and colour of the eye. Cream bambara groundnut seeds with black eye colour were selected for this study. Uniform seed weight (77.54 g/100 seeds) and seed dimensions [(length 1.23 cm) × width (1.01 cm) × height (0.94 cm)] were used. The cleaned seeds were packed in air tight container and stored at 4 ^{o}C until the time of use.

4.2.2.2 Methods

Hydrothermal process

The bambara groundnut seeds (100 g) were pre-soaked for 24 h at ambient temperature (25 °C) to 53% moisture level. Dehulling of pre-soaked bambara groundnut seeds was done manually by removing the seed coats after soaking. A tabletop microniser with 2 kW Phillips IR lamps (Technilamp Pty, Johannesburg, South Africa) was used for the current work. The microniser was first pre-heated for 20 min. The pre-soaked whole and dehulled samples were then micronised for 5, 10 and 15 min. After micronisation, the seeds were dried in a hot air dryer at 50 °C for 12 h until moisture contents of 7-11% were reached. The samples were then cooled to room temperature for 1h. The micronised seeds were milled to pass through a 500 µm aperture sieve using a Retsch ultra centrifugal mill (Model ZM200, Haan, Germany). The flours were stored in air tight container and kept at 4 °C until further analyses.

4.2.2.3 Analyses

Pasting properties

The pasting properties of bambara groundnut flours from unmicronised and micronised (whole and dehulled) pre-soaked bambara groundnut seeds were determined using a Rapid Visco Analyser (Model 3D, Newport Scientific Pty Ltd, Warriewood, Australia). Suspensions of flour (3.0 g) in dry basis were prepared in 25 mL of deionised water. The suspension was equilibrated at 50 °C for 1min and then heated to 91 °C at a uniform rate of 5 °Cmin^{-1} with constant stirring at 160 *rpm*. The heated slurry was held at 91 °C for 7min, then cooled to 50 °C at 5 °Cmin^{-1} and held at this temperature for 2 min.

Flour water solubility index and swelling power

The solubility of bambara groundnut flours from unmicronised and micronised (whole and dehulled) pre-soaked bambara groundnut seeds were determined using the method described by Ocloo, Minnaar and Emmambux (2014). About 0.125 g (dry basis) of flour samples were heated in 20 mL of distilled water at 50, 70, and 95 °C for 30 min in shaking water bath (100 *rpm*). The samples were then allowed to cool and centrifuged at 3000 × *g* for 15 min at 25 °C. The supernatant was decanted and evaporated in an air-oven at 105 °C for 16 h. The solubility was determined as the ratio of weight of dried supernatant to the weight of the flour and expressed as

percent (%). The residue obtained after centrifugation was then weighed to obtain the swelling power. Swelling power was expressed based on the weight of flour used.

Nitrogen solubility index

Nitrogen solubility index of the bambara groundnut flours from unmicronised and micronised (whole and dehulled) pre-soaked bambara groundnut seeds were determined according to the AACC Method 46-23 (AACC, 2000) with modification. About 1 g flour samples were dispersed in 20 mL of 0.1 M NaCl solution and stirred continuously for 1 h at 30 °C at pH 7. The suspension was centrifuged (9154.3 *g*, 15 min, and 4 °C) and the supernatant filtered through a Whatman No. 1 filter paper. The residue from the suspension was re-washed twice in 10mL of 0.1 M NaCl solution at pH 7. The filtrate was frozen (-18 °C) over night and freeze-dried (13KL, Instruvac Lyophilizer, Midrand, South Africa) for 4 days. The nitrogen content of the freeze-dried sample was determined using a Dumatherm (DT, Gerhardt Konigswinter, Germany). Nitrogen solubility index was expressed as a percentage of the total nitrogen content of freeze-dried sample divided by total nitrogen content in flour sample on a dry basis.

Thermal properties

The thermal properties of bambara groundnut flours from unmicronised and micronised (whole and dehulled) pre-soaked bambara groundnut seeds were evaluated according to the method reported by Ji et al. (2003) with modification. Flour samples were mixed with water in the ratio 2:1 (w/v), equilibrated for at least 12 h at ambient temperature and heated in a sealed Aluminium steel pan. A high-pressure DSC system with STARe® (HPDSC827^e, Mettler Toledo, Greifensee, Switzerland) was used to scan the sample at a rate of 10 $^oCmin^{-1}$ from 30 to 110 °C. Indium (T_o=156.0 °C, heat flow=-28.6 Jg^{-1}) was used as a standard to calibrate DSC and an empty pan as a reference. The following thermal parameters were measured: melting enthalpy (ΔH, J/g), onset temperature (T_o, °C), peak temperature (T_p, °C) and end temperature (T_c, °C) of any observed endotherm.

Light microscopy (LM)

Light microscopy (LM) of bambara groundnut flours from unmicronised and micronised (whole and dehulled) pre-soaked bambara groundnut seeds were performed. About 5.0mg (dry basis) of flour sample was dissolved in 1 mL of 30% glycerol solution. The flour suspensions were also

stained with and without iodine solution. A quick paste was also formed by mixing about 0.1 g (dry basis) of flour in 0.9 mL of boiling distilled water and heated for 5min. About 0.3 mL of 30% glycerol solution was added to the suspension and mixed. Samples (pasted and unpasted) were observed using a Nikon Optiphot Light Microscope (Tokyo, Japan) fitted with appropriate illumination sources and filters for normal with iodine staining and cross polarised; and pictures were captured with a Nikon digital camera DXM1200 (Tokyo, Japan).

Confocal laser scanning microscopy (CLSM)

About 0.1 g of bambara groundnut flours from unmicronised and micronised (whole and dehulled) pre-soaked bambara groundnut seeds were dissolved in 0.9 mL distilled boiling water and held in the water bath at boiling temperature for 5min to paste the sample. Two drops of 0.1% Safranin O dye (Sigma- Aldrich, St. Louis, MO, USA) was added to the paste. Two drops sample was placed on a concave microscope slide and covered with a cover slip. A Zeiss LSM 510 META Confocal Laser Scanning Microscope (Zeiss SMT, Jena, Germany) was used to observe and capturing of images of the samples. Plane neoflar100x and Numerical aperture (N.A) 1.4 was used for the blend images. The pixel time for both tracks 1 and 2 were 12.8 µs and picture size was 512 x 512 pixels. The excitation and emission spectra for the Safranin O dye were 488 nm and 540 nm, respectively.

Statistical analyses

The experimental work was repeated three times and the data was analysed using Statistica version 11 (StatSoftInc, Tulsa, USA). Multi-factor analysis of variance (MANOVA) was performed on all the measured values at $p \leq 0.05$. Significant means were separated using the least significant difference (LSD) test at $p \leq 0.05$. Independent variables were micronisation time (0, 5, 10 and 15 min) and dehulling treatment. Dependent variables were the measured values.

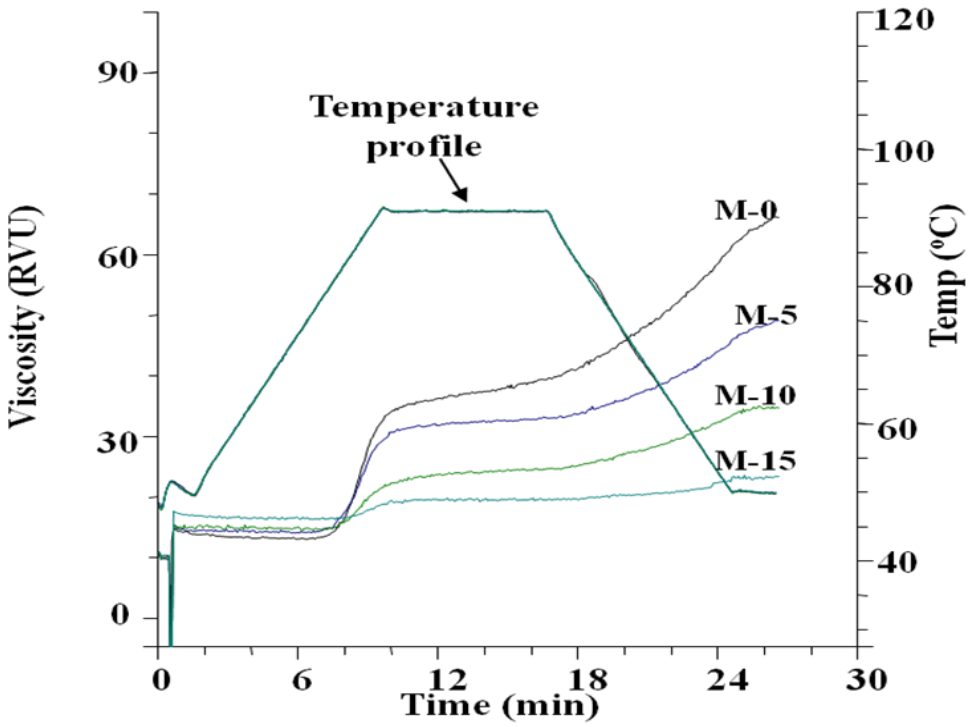
4.2.3 Results and Discussion

4.2.3.1 Pasting properties

Figure 4.7 shows the effects of micronisation time (0, 5, 10 and15 min) of whole and dehulled pre-soaked bambara groundnut seeds (53% moisture, 130 ºC) on the pasting viscosity of their resulting flours. The micronisation time and dehulling of bambara seeds had a significant ($p<0.001$) interaction on the pasting viscosity of resulting bambara flours. As the micronisation time (5, 10 and 15 min) increased, pasting viscosities of the resulting flours (both whole and dehulled) significantly ($p<0.001$) decreased. The pasting viscosity of flours from dehulled micronised pre-soaked bambara groundnut samples (10 and 15 min) were lower as compared to the flours from whole samples. The peak, final, setback and breakdown viscosities of flours from pre-soaked bambara micronised seeds (whole and dehulled) were significantly ($p<0.001$) lower than their unmicronised samples especially at 10 and 15 minutes.

Low pasting viscosity of flours from pre-conditioned (41% moisture) legume a seed such as a cowpea as a result of micronisation (130 and 170 ºC) has been reported (Mwangwela, Waniska, McDonough, & Minnaar, 2007a). However, it is noted that these authors pre-conditioned at 41% moisture rather than soaking cowpea and they did not use bambara groundnut which is bigger seeds in size. Bambara groundnut seeds are about 1.23 cm (length), 1.01 cm (width) and 0.94 cm (height) compared to cowpeas with size of about 0.92 cm (length), 0.65 cm (width) and 0.60 cm (height) (Kabas, Yilmaz, Ozmerzi, & Akinci, 2007). Mwangwela et al (2007a) hypothesised that the progressive decline in pasting viscosities of the flours with increasing micronisation temperature (especially at 170 ºC) could be due to reduction in swelling potential of physically induced crosslinked starch during subsequent gelatinisation or possibly starch depolymerisation, Besides these hypothetical suggestions the author did not make any further investigation to understand this phenomenon. Thus, various analyses relating to the starch, protein biomolecules and microstructure were done in the current work to understand the reduction in pasting viscosity of the resulting flours of micronised bambara groundnut seeds.

(A)



(B)

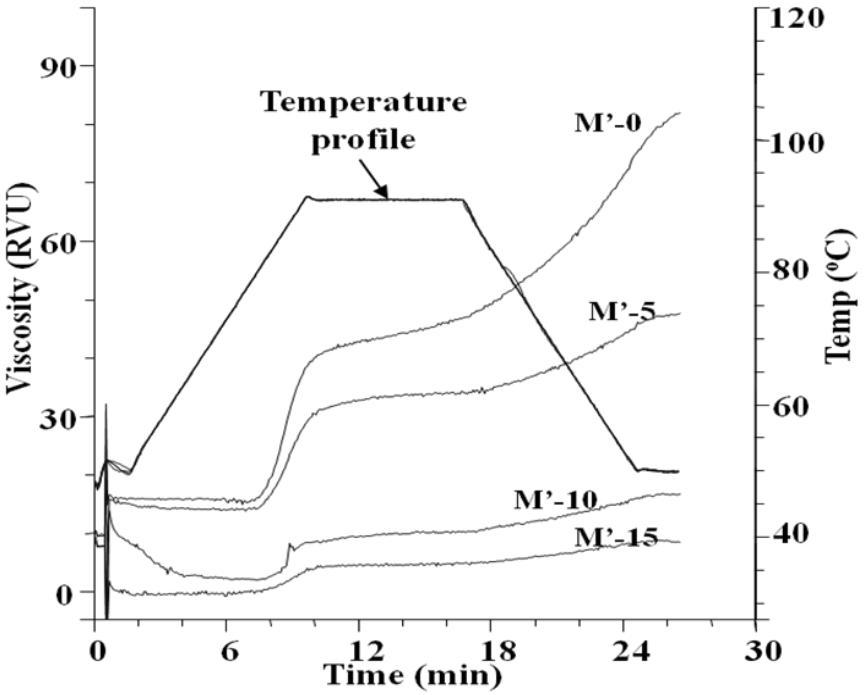


Figure 4.7 Effects of micronisation of whole (A) and dehulled (B) pre-soaked bambara groundnut seeds on the pasting properties of their resulting flours.
Whole flours (A): Unmicronised (M-0), Micronised:M-5 (5 min), M-10 (10 min), M-15 (15 min). Dehulled flours (B): Unmicronised (M'-0), Micronised:M'-5 (5 min)), M'-10 (10 min), M'-15 (15 min).

4.2.3.2 **Flour water solubility index and swelling power**

Swelling index of flours can be regarded as a measure of starch granule swelling as a result of gelatinisation and pasting; and water retention due to protein gelation (Mwangwela et al., 2007b). The effects of micronisation time (0, 5, 10 and 15 min) of whole and dehulled pre-soaked bambara groundnut seeds (53% moisture, 130 ºC) on the starch granules swelling of their resulting flours in water at different temperatures (50, 70 and 95 ºC) are shown in Table 4.3. The swelling index of starch granules in bambara groundnut flours from unmicronised and micronised samples (both whole and dehulled) significantly increased ($p<0.001$) with an increase in temperatures (50, 70 and 95 ºC). The starch granules in flours from bambara groundnut with an increase in micronisation time (5, 10 and 15 min) shows a slight increase in swelling index at 50 and 70 ºC. Notably at 95 ºC, the swelling index of starch granules in bambara groundnut flours from micronised samples significantly increased ($p<0.001$) by 4, 37 and 41% in whole samples and 22, 47 and 42% in dehulled samples with increasing micronisation time (5,10 and 15 min) respectively. Mwangwela et al. (2006) found pre-gelatinisation of starch and denaturation of protein in pre-conditioned micronised whole cowpea. The changes in resulting flours from micronised bambara groundnut may lead to the improvement in their water uptakes and swelling by starch granules and other biomolecules during soaking.

Flours from dehulled pre-soaked bambara groundnut samples (unmicronised and micronised) have a higher rate of swelling as compared to the flours from whole samples. This could be due to the difference in their chemical composition such as dietary fiber (e.g., cellulose, pectins, waxes, gums and lignin). In legume, dietary fiber (cell wall materials) is highly present in seed coats of whole seeds compared to the cotyledon (Tosh & Yada, 2010). The dietary fiber could compete with the water uptakes and swelling of starch granules (Brennan & Cleary, 2007).Therefore, the absence of the seed coat in pre-soaked dehulled bambara groundnut seeds could be responsible for the higher rate of the swelling experienced in their resulting flours.

Water solubility index of flour measures the water soluble fractions of components such as proteins, sugars and pectin in flour (Mwangwela et al., 2007b). Table 4.3 also represents the effects of micronisation time (0, 5, 10 and 15 min) of pre-soaked whole and dehulled bambara groundnut seeds (53% moisture, 130 ºC) on the solubility of the resulting flours in water measured at different temperatures (50, 70 and 95 ºC).

Table 4.3 Effects of micronisation of whole and dehulled pre-soaked bambara groundnut seeds on the water solubility and swelling index of their resulting flours

Bambara groundnut samples	Micronisation time (min)	Measuring temp. (ºC)	WSI (%)	Swelling power (g/g)
Whole	0	50	28.8^{jkl} (0.4)[1]	2.1^{a} (0.03)
		70	27.9^{j} (2.0)	2.3^{abc} (0.2)
		95	31.0^{mn} (0.5)	4.6^{jk} (0.1)
	5	50	28.0^{jk} (0.4)	2.2^{ab} (0.1)
		70	27.5^{ij} (0.4)	2.6^{cd} (0.1)
		95	28.8^{jkl} (2.0)	4.8^{k} (0.3)
	10	50	21.7^{cde} (0.5)	2.8d^{e} (0.1)
		70	22.6^{ef} (0.3)	3.1e^{fg} (0.6)
		95	24.0^{fg} (2.0)	6.3^{m} (0.3)
	15	50	18.6^{a} (0.7)	3.5^{h} (0.1)
		70	17.7^{a} (0.3)	4.0^{i} (0.1)
		95	20.3^{bc} (1.3)	6.5^{m} (0.2)
Dehulled	0	50	29.7^{lm} (0.2)	2.0^{a} (0.1)
		70	32.0^{no} (0.6)	2.0^{a} (0.1)
		95	32.5^{o} (0.4)	4.5^{jk} (0.1)
	5	50	25.0^{gh} (0.1)	2.5^{bcd} (0.1)
		70	26.1^{hi} (0.5)	2.9^{ef} (0.1)
		95	29.4^{klm} (1.1)	5.5^{l} (0.04)
	10	50	21.1^{bcd} (0.8)	3.3^{fgh} (0.1)
		70	21.3^{bcde} (1.0)	3.5^{h} (0.2)
		95	22.5^{de} (1.1)	6.6^{m} (0.01)
	15	50	20.5^{b} (1.2)	3.2^{fgh} (0.1)
		70	20.2^{bc} (0.2)	4.4^{j} (0.4)
		95	20.2^{b} (0.2)	6.4^{m} (0.1)

WSI: Water solubility index. [1]Means Standard deviation n=3. Values with similar superscripts in rows are not significantly different (p< 0.001) Whole samples: Unmicronised (0), Micronised: 5 (5 min), 10 (10 min), 15 (15 min). Dehulled samples:Unmicronised (0),Micronised: 5 (5 min), 10 (10 min), 15 (15 min).

The water solubility index in bambara groundnut flours from unmicronised and micronised (both whole and dehulled) samples significantly increased ($p<0.001$) with an increase in temperatures (50, 70 and 95 ºC). The water solubility index of flours (whole and dehulled samples) from bambara groundnut at 50 and 70 ºC slightly decreased with the increase in micronisation time. The water solubility index of resulting bambara groundnut flours at 95 ºC significantly ($p<0.001$) reduced by 7, 22 and 35% in whole and 10, 31 and 38% in dehulled samples as the micronisation time increased to 5, 10 and 15 min. This reduction in the water solubility index could be due to the denaturation of the protein in the resulting flours of pre-soaked micronised (whole and dehulled) bambara groundnut as a result of the micronisation. Other authors have found that micronisation causes denaturation of protein of legume seeds (Mwangwela et al. 2007b; Fasina et al. 2001) and these led to the reduction in the protein solubility of the resulting flours from the pre-conditioned micronised legume seeds such as cowpea (Mwangwela et al. 2007b). In order to confirm this, the nitrogen solubility index of the resulting flours of pre-soaked micronised whole and dehulled bambara groundnut samples was determined.

4.2.3.3 **Nitrogen solubility index (NSI)**

The effects of micronisation time of whole and dehulled bambara groundnut seeds on the nitrogen solubility of their resulting flours are shown in Table 4.4. An increase in micronisation time (5, 10 and 15 min) significantly ($p<0.001$) reduced the nitrogen solubility of bambara groundnut flours from both whole (133, 76 and 42%) and dehulled (138, 91 and 42%) seeds, respectively (Table 4.4).This reduction in the nitrogen solubility with an increase in micronisation time showed that protein in the bambara groundnut flours from micronised samples could less soluble and more hydrophobic. Mwangwela, Waniska, & Minnaar (2007b) and Zheng, Fasina, Sosulski, & Tyler, (1998) have reported similar a reduction in nitrogen solubility of legumes flours upon micronisation of the pre-conditioned seeds and these authors suggested denaturation of protein and exposure of hydrophobic amino acids. Isolated protein from kidney, red and mung beans became more hydrophobic when heat processed at 95 ºC for 30 min (Tang, Sun & Yin, 2009).

The reductions in water solubility could possibly be due to the reduction in solubility of the protein of resulting flour (Table 4.4) from pre-soaked and micronised whole and dehulled bambara groundnut seeds. Mwangwela, Waniska, & Minnaar (2007b) also attributed the

reduction of water solubility in cowpea flours from micronised pre-conditioned cowpea seeds (41% moisture, 130 and 170 ºC respectively) to the reduction in solubility of the protein.

Table 4.4 Effects of micronisation of whole and dehulled pre-soaked bambara groundnut seeds on the Nitrogen solubility index (dry basis) of their resulting flours

Micronisation time (min)	Whole samples NSI (%)	Dehulled samples NSI (%)
0	134.6^{de} (1.1)[1]	142.3^{f} (1.6)
5	132.5^{d} (1.7)	138.1^{e} (1.0)
10	76.3^{b} (2.0)	90.6^{c} (0.1)
15	42.0^{a} (0.8)	42.2^{a} (2.6)

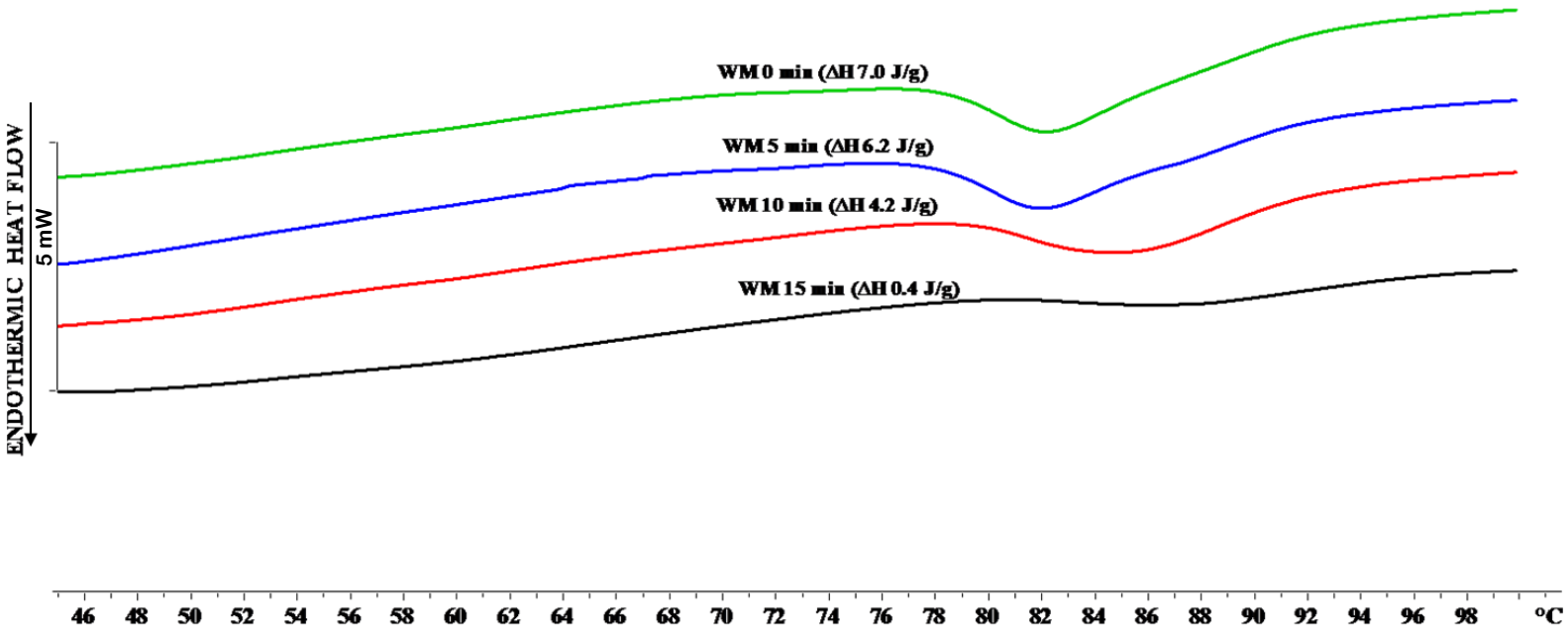
[1]*Means Standard deviation n=3. Values with similar superscripts in rows are not significantly different (p< 0.001) Whole samples: Unmicronised (0), Micronised: 5 (5min), 10 (10 min), 15 (15 min). Dehulled samples: Unmicronised (0), Micronised: 5 (5 min), 10 (10 min), 15 (15min)*

4.2.3.4 **Differential Scanning Calorimetry (DSC)**

Figure 4.8 represent the effects of micronisation time (0, 5, 10 and 15 min) on the thermal property of their resulting flours from the whole and dehulled pre-soaked bambara groundnut seeds (53% moisture, 130 ºC). The bambara groundnut flour (whole and dehulled) samples exhibited a single thermal transition at about 77.0- 78.4 ºC Onset (T_o), 82.0- 87.3 ºC Peak (T_p) and 86.1- 93.0 ºC Endpoint (T_c).This single endothermic peak is likely to be due to starch gelatinisation. Starch gelatinisation of bambara groundnut was reported to be at 76.8 ºC Onset (T_o), 80.7 ºC Peak (T_p) and 85.8 ºC Endpoint (T_c) (Sirivongpaisal, 2008).

Micronisation time significantly ($p<0.001$) reduced the endothermic peak of the flours from both micronised whole and dehulled pre-soaked bambara groundnut samples compared to unmicronised samples. The enthalpy of the endothermic peak significantly ($p<0.001$) reduced in flours from micronised bambara groundnut seeds by 11, 40 and 94% (whole samples) and 10, 65 and 93% (dehulled samples) respectively for 5, 10, and 15 min micronisation time. Flours from dehulled micronised bambara groundnut samples in terms of micronisation time have a higher rate of decrease in the enthalpy of the endothermic peak as compared with the whole samples.

(A)


WM 0 min (ΔH 7.0 J/g)
WM 5 min (ΔH 6.2 J/g)
WM 10 min (ΔH 4.2 J/g)
WM 15 min (ΔH 0.4 J/g)
ENDOTHERMIC HEAT FLOW
5 mW
46 48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 82 84 86 88 90 92 94 96 98 °C

(B)

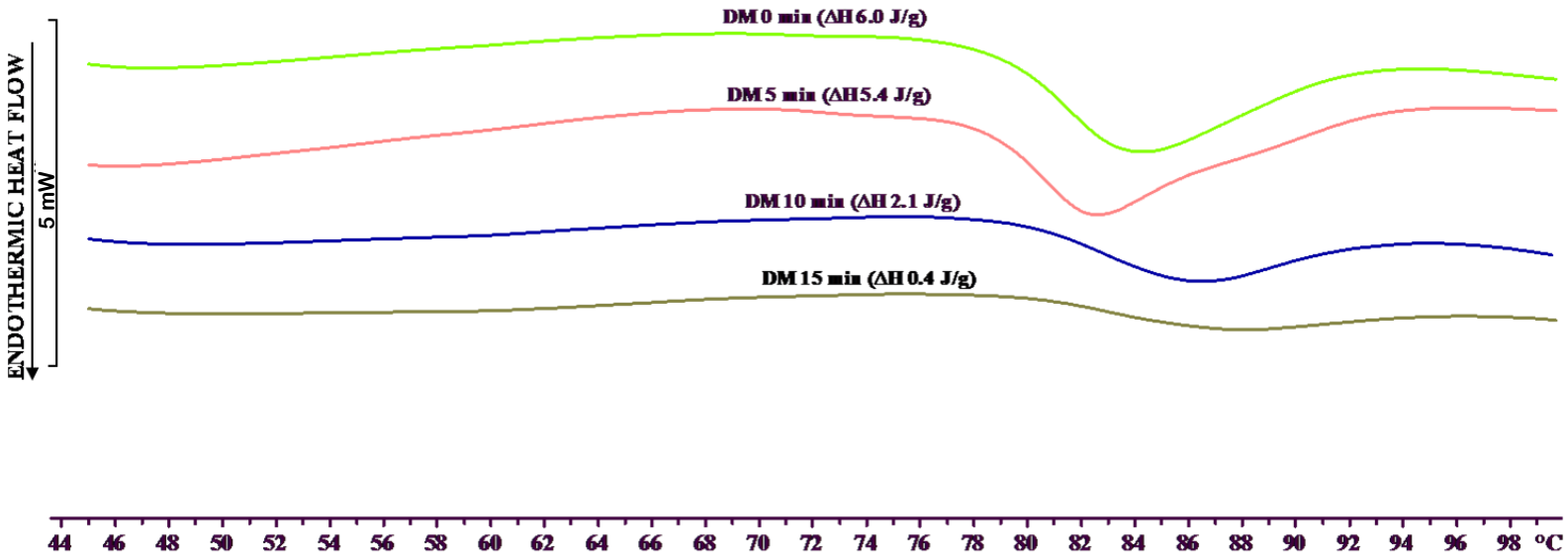


Figure 4.8 Effects of micronisation of whole (A) and dehulled (B) pre-soaked bambara groundnut seeds on the thermal properties of their resulting flours

In bracket are enthalpies of whole flours (A): WM0 min (Unmicronised whole flour); WM5 min, WM10 min and wm15 min (Micronised whole flours from whole bambara seeds at 5, 10 15 minute. In bracket are enthalpies of dehulled flours (B): DM0 min (Unmicronised dehulled bambara flour); DM5 min, DM10 min and DM15 min (Micronised dehulled flours from dehulled bambara seeds micronised at 5, 10 15 minutes)

These suggest that starch granules of dehulled samples (micronised) gelatinised faster by losing their integrity as compared to the whole samples during micronisation. Mwangwela, Waniska, McDonough, and Minnaar (2007a) previously reported a reduction in enthalpy for an endothermic peak of cowpea flours from micronised seeds (41% moisture, 130 and 170 ºC). They suggested a change in the crystalline order and nature of the starch granules in the flour from the resulting micronised cowpea seeds. The decrease in the enthalpy of bambara flours from micronised samples as the micronisation increased may suggest that the starch molecules were partially gelatinised and this increased with the increase in micronisation time.

4.2.3.5 **Microscopy (LM & CLSM)**

The bambara groundnut flours (unmicronised and micronised) under polarised light microscopy (Figure 4.9) confirmed the contribution of pre-gelatinisation of starch in flours from micronised samples due to the reduction of the endothermic peak. The reduction in birefringence of starch was due to effects of micronisation for both whole and dehulled bambara groundnut seeds. The amount of birefringence in flours from micronised pre-soaked seeds (whole and dehulled) decreased with increase in micronisation time. This shows that more starch granules were gelatinised due to the disappearance of Maltese crosses as the micronisation time increased. Dehulled flour samples (10 and 15 min) show more loss of birefringence indicating more pre-gelatinisation of starch in the flours. Reduction in birefringence has also been reported in flours from micronised cowpea (41% moisture, 130 and 170 ºC) (Mwangwela et al. 2007a) and hydrothermally treated pea, lentil and navy bean starches (Chung, Liu, & Hoover, 2010). This shows that starch granules from bambara groundnut pre-gelatinised when hydrothermally treated by micronisation.

Figure 4.10 is micrographs of iodine-stained flours (whole and dehulled samples) from pre-soaked bambara groundnut seeds. It was observed that the starch granules in unmicronised samples (whole and dehulled) are smaller as compared to their micronised samples and the starch granules individually bind with the iodine stain. As the micronisation time increased (0, 5, 10 and 15 min) in both whole and dehulled micronised samples, the swelling of the starch granules also increases. The starch granules in flours (whole and dehulled) from micronised samples were increasingly coming together to form aggregates with an increase in micronisation time as observed in Fig 4.10.

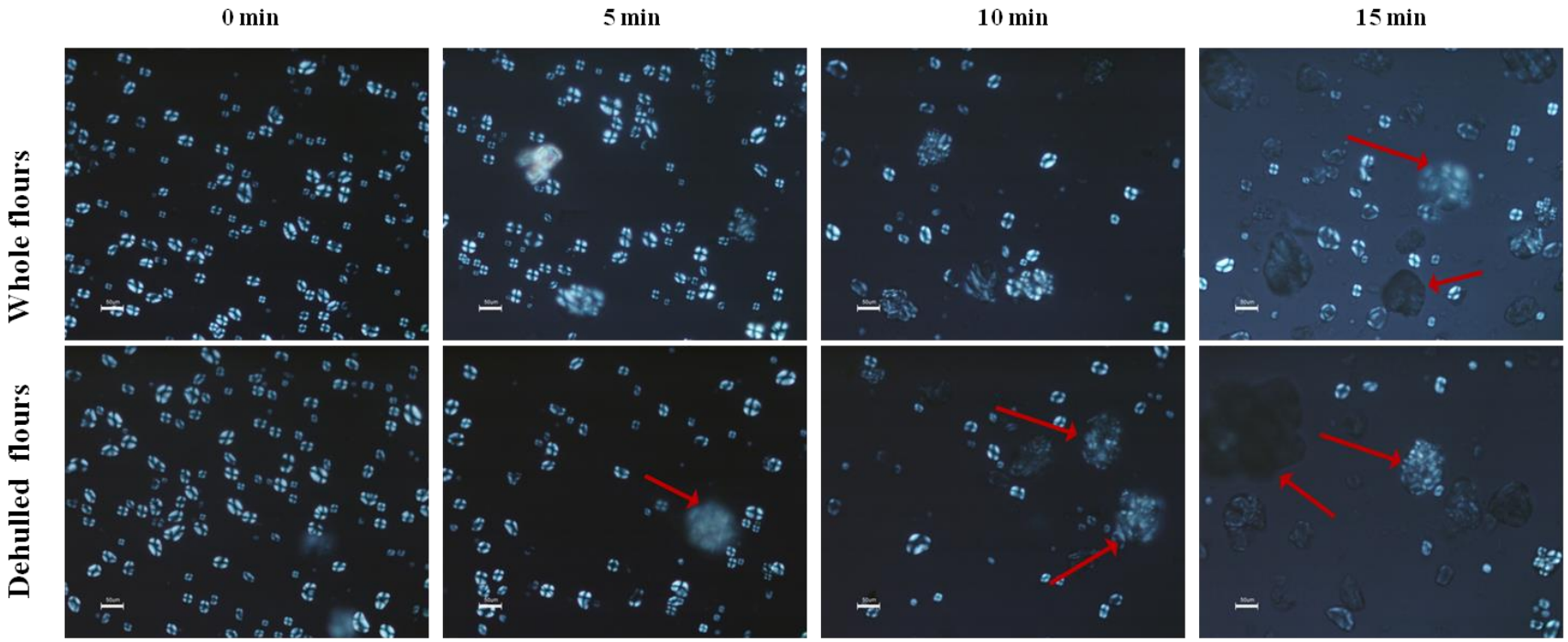


Figure 4.9 Polarised light micrographs of flours from pre-soaked micronised bambara groundnut

Bambara flours made from micronised whole and dehulled seeds (0, 5, 10 and 15 min) were viewed under polarised light for loss of birefringence. Bar = 50 µm. Arrows indicating aggregates in flours from micronised samples. Micronisation time increased reduced birefringence of starch granules in flours of micronised samples.

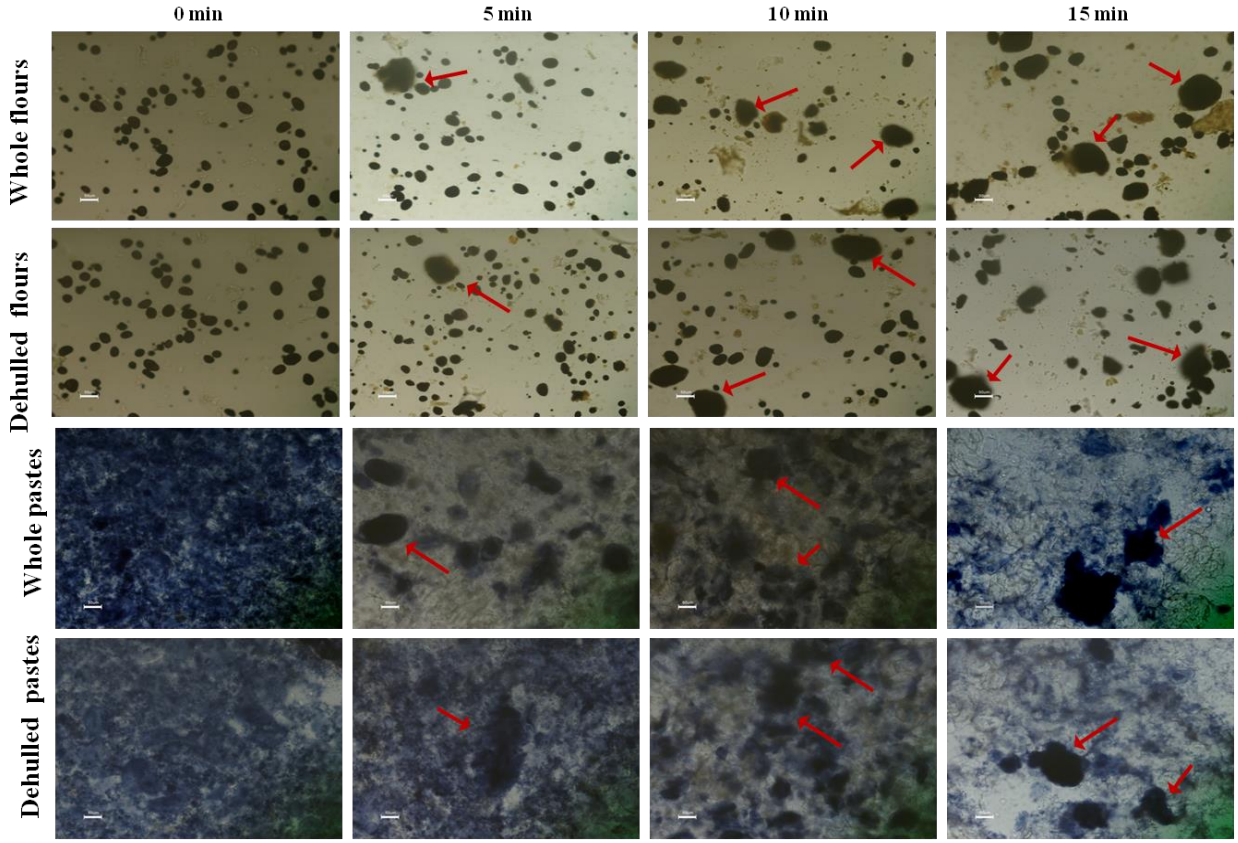


Figure 4.10 Light micrographs of iodine stained flours and pastes from pre-soaked micronised bambara groundnut samples

Bambara flours and pastes made from micronised whole and dehulled samples (0, 5, 10 and 15min) were stained for starch with iodine solution. Bar = 50µm. Arrows indicating Aggregates in micronised flours and pastes. Increase in micronisation time increased the aggregates formation reduced the iodine staining of starch in the dispersions of micronised flour samples.

The iodine stained micrographs of pasted samples from pre-soaked unmicronised and micronised bambara groundnut seeds (whole and dehulled samples) were also shown in Figure 4.10. The micrographs show that there was no starch granular evidence in the pastes formed from unmicronised flours sample.The starches were well dispersed in the pastes of unmicronised whole and dehulled samples but there were notable evidence of increasing aggregation observed in the flours from both (whole and dehulled) micronised samples as micronisation time (0, 5, 10 and 15 min) increased. The extent of iodine staining by starch granules in the dispersions (pastes) of micronised flour (whole and dehulled samples) reduced with an increase in micronisation time compared to the unmicronised samples. The non-stained samples were more in the aggregated particles. The reduction of iodine staining indicates that the iodine could not bind with the amylose to show the presence of starch or the stain could not effectively diffuse to reach

amylose. The aggregated particle can be due to protein and starch interaction. As the protein became more hydrophobic due to micronisation as shown by nitrogen solubility index (Table 4.4), this could prevent the iodine stains reaching the starch amylose molecules.

To further understand these aggregates in micronised samples, the pastes of bambara flours from the whole and dehulled samples (unmicronised and micronised) stained with safranin O dye were viewed using confocal laser scanning microscopy (Figure 4.11). Safranin O dye preferentially stains proteins. CLSM micrographs as indicated by the arrows also show aggregation in the pastes from micronised samples compared to their unmicronised ones as shown with light microscopy (Figure 4.10). Fig 4.11 as observed (indicated by the arrow) suggested that aggregate is made up of unstained black granular structure embedded by a red stained matrix. The stained (red spots) in the aggregate is most likely to be protein matrixes as safranin dye stain protein (Moore, Schober, Dockery & Arendt, 2004) and the unstained (black spots) of the aggregate could be the starch granular structures embedded in denatured protein matrix. The increased in micronisation time of pre-soaked bambara groundnut (whole and dehulled) increase extent of denaturation in the protein causing protein unfolding and exposure of hydrophobic sites. As the starch is surrounded by the hydrophobic protein matrix, starch granules do not swell during wet heat processing due to limited water diffusion into the starch granules.

Protein denaturation contributed to reduction in nitrogen solubility of flours from pre-conditioned micronised cowpea (Mwangwela et al. 2007b) and peas (Cenkowski & Sosulski, 1998) and other grains (Bellido, Arntfield, Cenkowski, & Scanlon, 2006; Arntfield et al. 2001; Zheng, Fasina, Sosulski, & Tyler, 1998). The denaturation of water and salt soluble protein (albumin and globulins respectively) were reported to be more pronounced in flours from micronised pre-conditioned legume seeds compared with the unmicronised (Zheng et al. 1998). The hydrothermal treatment probably induced in proteins crosslinking through electrostatic and disulphide interactions (Tang & Ma, 2009) to strengthen the protein matrix; as well as exposing hydrophobic groups and this reduces the solubility of the protein in water (Ashraf, Saeed, Sayeed, & Ali, 2012; Zheng et al. 1998). Heat treatment at the temperature close or higher than the denaturation temperature (95ºC) of vicilin (globular protein) in kidney beans protein isolate was reported to form aggregates due to electrostatic repulsive interaction (Tang & Ma, 2009).

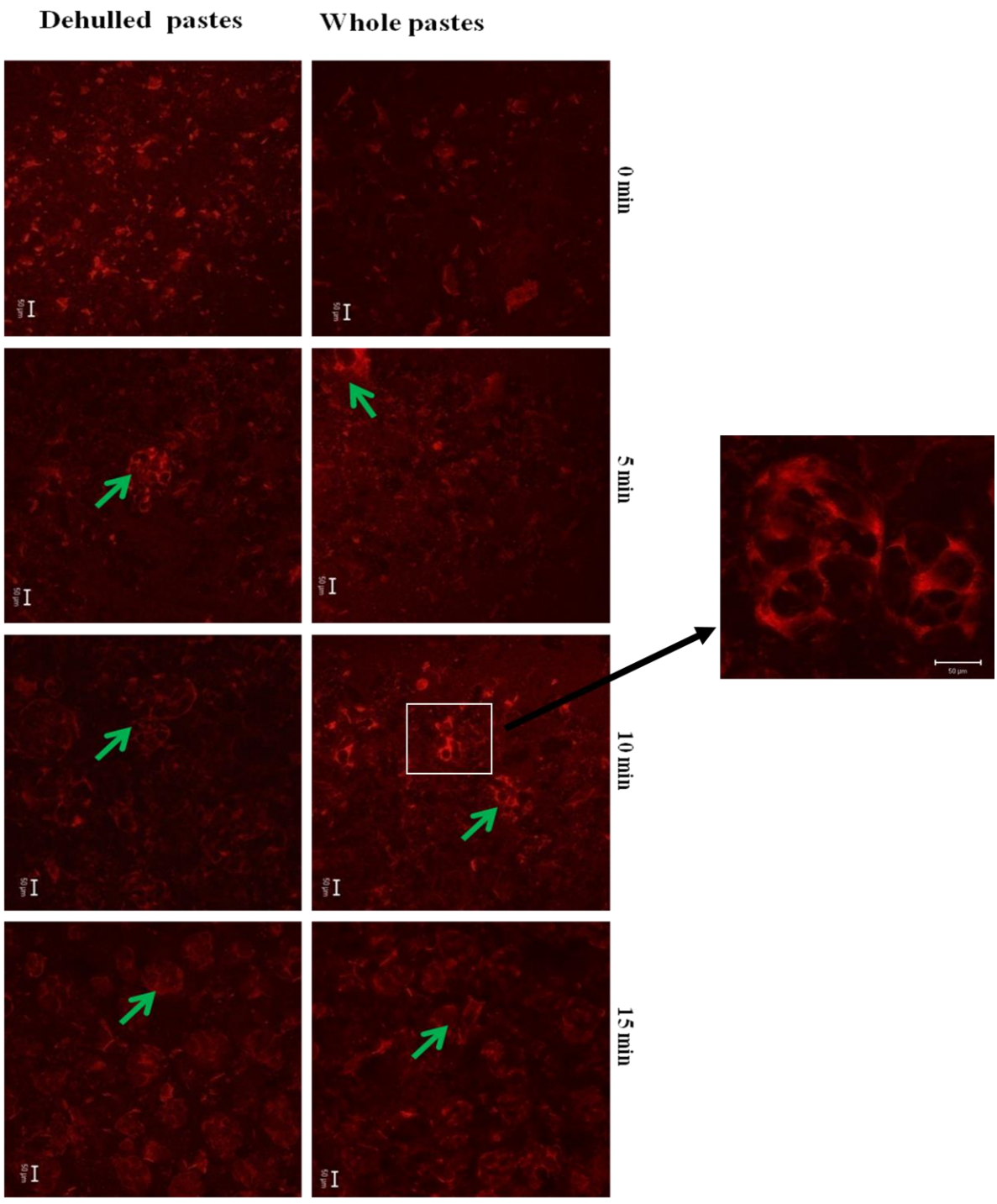


Figure 4.11 Confocal laser scanning micrographs of pastes from whole and dehulled pre-soaked bambara groundnut

Pastes were produced from bambara flours made from micronised whole and dehulled seeds (0, 5, 10 and 15min) and stained for protein with Safranin O dye. Bar = 50µm. Arrows indicating aggregation in pastes of micronised flour samples. Black spots inside the red stained indicates the starch and the red indicates protein matrix

These authors also reported heat treatment (95 ºC) could account for the increase in exposure of buried hydrophobic globular protein residue during thermal denaturation. The exposure of these hydrophobic amino acid sites of the denatured protein in resulting flours of micronised pre-soaked bambara groundnuts is likely to be responsible for repelling of water away by the starchgranules embedded in the protein matrix. These could also be responsible for the reduction water solubility index and pasting viscosity of the flours from micronised samples.

Dehulled samples seem to have a higher effect than whole samples as can be seen from results obtained from pasting viscosity (Fig. 4.7), swelling index (Table 4.3), water solubility index (Table 4.3), nitrogen solubility index (Table 4.4), enthalpy of endothermic peak (Fig. 4.8) and micrographs (Figs. 4.9, 4.10 and 4.11) of the flour samples from bambara groundnut. This could be attributed to the absence of seed coats in dehulled samples and because the dehulling pre-treatment of pre-soaked bambara groundnut results in split seeds which enhance further processing (micronisation) than whole samples. Also, the presence of the non-starch polysaccharides such as dietary fibre in the seed coats could also contribute to the less effect of the results obtained compared to the dehulled samples. The dehulling of seed coat of legume seeds has been reported to increase protein content (Nti, 2009) and reduced non-starch polysaccharides (dietary fibre) (Wang et al. 2009) of the legume seeds such as lentils and bambara groundnut. Penicela (2011) found that the seed coat removal from different varieties of cowpea was attributed to the improvement in cooking characteristics such as water absorption, cooking time and texture of dehulled samples during soaking and cooking as compared to whole samples. The splitting of the cotyledon into two halves reduced seeds size and will provide an increase in surface area of the bambara groundnut in dehulled samples than whole samples. Cenkowski, Arntfield, & Scanlon, (2007), reported that the thickness of a material could influence the depth of penetration of infrared radiation energy through the material. With the absence of seed coats and splitting occurring in the dehulled samples, the penetration of infrared radiation will reach more biomolecules compared to whole grain samples. Thus this result in higher effect in functionality compared to whole grain.

4.2.4 Conclusions

Micronisation of pre-soaked bambara groundnut causes the reduction in pasting viscosity of resulting flours. The low pasting viscosity phenomenon seems to be due to the microstructural changes of bambara groundnut as a result of micronisation as protein was denatured and the starch was gelatinised during micronisation. Micronisation denatures protein in the bambara groundnut and this exposes the hydrophobic sites. This could be in part prevents starch hydration and dispersion during pasting and thus reduced viscosity. Dehulling prior to micronisation reduced the pasting viscosity of the resulting flour more compared to the whole samples, suggesting a higher effect of micronisation for dehulled samples. Resulting Flours from micronised bambara groundnut can be useful ingredients in protein energy-dense foods due to their low viscosity.

4.2.5 References

American Association of Cereal Chemists, AACC, 2000. *Approved methods of the AACC* (10th ed.) St. Paul, MN: USA.

Afoakwa, E. O., Budu, A. S., & Merson, A. B., 2007. Application of response surface methodology for optimizing the pre-processing conditions of bambara groundnut *(Voandzeian subterranea)* during canning. *International Journal of Food Engineering*, 2.

Alozie, Y. E., Iyam, M. A., Lawal, O., Udofia, U., & Ani, I. F., 2009. Utilization of bambara groundnut flour blends in bread production. *Journal of Food Technology*, 7, 111-114.

Aremu, M., Olaofe, O., & Akintayo, E., 2007. Functional properties of some Nigerian varieties of legume seed flours and flour concentration effect on foaming and gelation properties. *Journal of Food Technology*, 5, 109-113.

Arntfield, S. D., Scanlon, M. G., Malcolmson, L. J., Watts, B. M., Cenkowski, S., Ryland, D. & Savoie, V. 2001. Reduction in lentil cooking time using micronisation: Comparison of 2 micronisation temperatures. *Journal of Food Science*, 66, 500-505.

Ashraf, S., Saeed, S. M. G., Sayeed, S. A., & Ali, R., 2012. Impact of microwave treatment on the functionality of cereals and legumes. *International Journal of Agriculture and Biology*, 14, 365-370.

Barimalaa, I. S., Agoha, G., Oboh, C. A., & Kiin-Kabari, D. B., 2005. Studies on bambara groundnut flour performance in okpa preparation. *Journal of the Science of Food and Agriculture*, 85, 413-417.

Bellido, G., Arnt-field, S. D., Cenkowski, S., & Scanlon, M., 2006. Effects of micronization pretreatments on the physicochemical properties of navy and black beans *(Phaseolus vulgaris L.).LWT - Food Science and Technology*, 39, 779-787.

Brennan, C. S., & Cleary, L. J., 2007. Utilisation glucagel® in the β-glucan enrichment of breads: A physicochemical and nutritional evaluation. *Food Research International*, 40, 291-296.

Chung, H. J., Liu, Q., & Hoover, R., 2010. Effect of single and dual hydrothermal treatments on the crystalline structure, thermal properties, and nutritional fractions of pea, lentil, and navy bean starches. *Food Research International*, 43, 501-508.

Cenkowski, S., & Sosulski, F., 1998. Cooking characteristics of split peas treated with infrared heat. *Transactions of the ASAE*, 41, 715-720.

Cenkowski, S., Arntfield, S., & Scanlon, M., 2007. Far infrared dehydration and processing. *Food Drying Science and Technology: Microbiology, Chemistry, Application*, 157.

Eltayeb, A. R. S., Ali, A. O., Abou-arab, A. A., & Abu-Salem, F. M., 2011. Chemical composition and functional properties of flour and protein isolate extracted from bambara groundnut *(Vigna subterranean). African Journal of Food Science*, 5, 82-90.

Fasina, O., Tyler, B., Pickard, M., Zheng, G.H., & Wang, N., 2001. Effect of infrared heating on the properties of legume seeds.*International Journal of Food Science & Technology*, 36, 79-90.

Ji, Y., Seetharaman, K., Wong, K., Pollak, L., Duvick, S., Jane, J., & White, P., 2003. Thermal and structural properties of unusual starches from developmental corn lines. *Carbohydrate Polymers*, 51, 439-450.

Kabas, O., Yilmaz, E., Ozmerzi, A., & Akinci, İ., 2007. Some physical and nutritional properties of cowpea seed *(Vigna sinensis L.). Journal of Food Engineering*, 79, 1405-1409.

Kayitesi, E., Duodu, K. G., Minnaar, A., & De Kock, H. L., 2012.Effect of micronisation of pre-conditioned cowpeas on cooking time and sensory properties of cooked cowpeas.*Journal of the Science of Food and Agriculture*, 93, 838-845.

Mwangwela, A. M., Waniska, R. D., & Minnaar, A., 2006. Hydrothermal treatments of two cowpea *(Vignaunguiculata L. Walp)* varieties: effect of micronisation on physicochemical and structural characteristics. *Journal of the Science of Food and Agriculture*, 86, 35-45.

Mwangwela, A. M., Waniska, R. D., Mcdonough, C., & Minnaar, A., 2007a. Cowpea cooking characteristics as affected by micronisation temperature: a study of the physicochemical and functional properties of starch. *Journal of the Science of Food and Agriculture*, 87, 399-410.

Mwangwela, A. M., Waniska, R. D., &Minnaar, A., 2007b. Effect of micronisation temperature (130 and 170º C) on functional properties of cowpea flour. *Food Chemistry*, 104, 650-657.

Moore, M. M., Schober, T. J., Dockery, P., & Arendt, E. K., 2004. Textural comparisons of gluten-free and wheat-based doughs, batters, and breads. *Cereal Chemistry*, 81, 567-575.

Nti, C. A., 2009. Effects of bambara groundnut *(Vigna subterranea)* variety and processing on the quality and consumer appeal for its products. *International Journal of Food Science & Technology*, 44, 2234-2242.

Ocloo, F. C., Minnaar, A., & Emmambux, N. M., 2014. Effects of gamma irradiation and stearic acid, alone and in combination, on functional, structural, and molecular characteristics of high amylose maize starch. *Starch-Stärke*, 66, 624-635.

Ojimelukwe, P. C., 1999. Cooking characteristics of four cultivars of bambara groundnut seeds and starch isolate. *Journal of Food Biochemistry*, 23, 109-117.

Penicela, L., 2011. The Influence of seed coat and cotyledon structure on cooking characteristics of cowpea.MSc dissertation.(Food Science).University of Pretoria.

Prinyawiwatkul, W., Beuchat, L., R., Mcwatters, K. H., & Phillips, R. D., 1997. Functional properties of cowpea *(Vigna unguiculata)* flour as affected by soaking, boiling, and fungal fermentation. *Journal of Agricultural and Food Chemistry*, 45, 480-486.

Rastogi, N. K., 2012. Recent trends and developments in infrared heating in food processing.*Critical Reviews in Food Science and Nutrition*, 52, 737-760.

Scanlon, M. G., Cenkowski, S., Segall, K. I., & Arntfield, S. D., 2005. The physical properties of micronised lentils as a function of tempering moisture. *Biosystems Engineering*, 92, 247-254.

Sirivongpaisal, P., 2008. Structure and functional properties of starch and flour from bambarra groundnut. *Sonklanakarin Journal of Science and Technology*, 30, 51.

Sharma, G., 2009. Micronisation.Defence Research and Development Organization, Ministry of Defence, Government of India. *Science Spectrum*, 169-171.

Tang, C.H., & Ma, C.Y., 2009. Heat-induced modifications in the functional and structural properties of vicilin-rich protein isolate from kidney *(Phaseolus vulgaris L.)* bean. *Food Chemistry*, 115, 859-866.

Tang, C.H., Sun, X., & Yin, S.W., 2009. Physicochemical, functional and structural properties of vicilin-rich protein isolates from three phaseolus legumes: Effect of heat treatment. *Food Hydrocolloids*, 23, 1771-1778.

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

Yusuf, A., Ayedun, H., & Sanni, L., 2008. Chemical composition and functional properties of raw and roasted Nigerian benniseed *(Sesam umindicum)* and bambara groundnut *(Vigna subterranean)*. *Food Chemistry*, 111, 277-282.

Wang, N., Hatcher, D., Toews, R., & Gawalko, E., 2009. Influence of cooking and dehulling on nutritional composition of several varieties of lentils *(Lens culinaris)*. *LWT-Food Science and Technology*, 42, 842-848.

Zheng, G., Fasina, O., Sosulski, F., & Tyler, R., 1998. Nitrogen solubility of cereals and legumes subjected to micronisation. *Journal of Agricultural and Food Chemistry*, 46, 4150-4157.

4.3 Nutritional and health benefits of cooked soft porridge from resultant flours of pre-soaked micronised bambara groundnut (*Vigna subterranea*) seeds

Abstract

Bambara groundnut is an underutilised crop grown in Africa. Its utilisation may be increased through value addition into its flour. Micronisation is a hydrothermal processing technology and has influenced the functionality of some other legumeseeds and their resulting flour. This study determines the effects of micronisation of pre-soaked whole and dehulled bambara groundnut samples (53%, 130 ºC) at 0, 5, 10 and 15 min on the nutritional, apparent viscosity, total extractable phenolics and antioxidant activity of cooked soft porridge from resulting flour. The *invitro* protein and starch digestibility of cooked whole and dehulled bambara groundnut samples significantly ($p<0.01$) increased with micronisationdue to protein denaturation and starch gelatinisation respectively, therefore increasing accessibility for the digestive enzymes. Whole and dehulled bambara groundnut samples showed a reduced apparent viscosity and hysteresis with micronisation. Total phenolic contents and radical scavenging properties ($ABTS^{+}$) in extracts of whole samples increased with micronisation upon cooking. This suggests that some phenolics of cooked whole micronised bambara groundnut samples becomes more extractable, possibly by release of bound ones. The absence of seed coat led to reduction in total phenolic contents and radical scavenging properties ($ABTS^{+}$) in extracts of dehulled samples. Resulting flour of micronised pre-soaked bambara groundnut could produce porridge with potential to improve the nutritional quality, nutrient density and health promoting property.

4.3.1 Introduction

Bambara groundnuts (*Vigna subterranean*) are underutilised legume belonging to the family of Fabaceae. Bambara groundnut grows well in low fertile soil conditions and it's mostly cultivated by female farmers for subsistence use in the arid parts of sub-Saharan Africa (Azam-Ali et al., 2001). It is also grown in various parts of South America and Asia (Azam-Ali et al., 2001). Bambara groundnut contributes to the nitrogen fixation of the soils; hence, it is intercropped along with other grains such as cowpea (Alhassan et al., 2012) and maize (Hillocks et al., 2012). Bambara groundnut is highly nutritious (Ouedraogo et al., 2008), for example, the flour contains about 29 to 30% protein on dry basis and it's rich in essential amino acids such as lysine and tryptophan (Adebowale et al., 2011). The flour from bambara groundnut has good functionality such as water binding, foaming, gelation, bulk density and emulsification (Onimawo et al., 1998; Eltayeb et al., 2011). Its good functional and protein quality makes its flour suitable as complementary products with cereal based grains such as wheat (Alozie et al., 2009) and sorghum (Lyimo, 2000; Nnam, 2001).

Bambara groundnuts could also have other health benefits in association with their consumption (Nyau et al., 2015). It contains bioactive compound such as phenolics which have antioxidant properties (Nyau et al., 2015; Jideani & Diedericks, 2014). Heat processing is useful in improving the nutritional (Siddhuraju & Becker, 2005; Rehman & Shah, 2005; Siddhuraju & Becker, 2001) and health promoting compound (Nithiyanantham et al., 2012; Xu & Chang, 2008a) of legumes.

Micronisation is a hydrothermal processing technology involving an application of infrared heating with pre-moisture conditioned grains (Kayitesi et al., 2012; Adrejko et al., 2008; Arntfield et al., 2001). The pre-conditioned micronised legume such as cowpea produced instantised flour through starch gelatinisation, protein denaturation and pectin solubilisation (Vilakati et al., 2015). In previous research chapters of the research, it was reported that micronisation-induced changes in structure and molecular properties of starch and protein was responsible for the improvement in hydration and swelling property of resulting flours. The low pasting viscosity phenomenon of resulting flours caused by micronisation of pre-soaked bambara groundnut seeds was due to the microstructural changes in the protein matrixes shown by confocal laser scanning microscopy. The exposure of hydrophobic sites could be in part prevents

starch hydration and dispersion during pasting and thus reduced viscosity. Thus the application of this resulting flour could have potentials in food system in development instant product. The combination of micronisation with extrusion cooking was reported to improve protein quality (lysine) and digestibility of cereal – legume based ready to eat porridge for children (Vilakati et al., 2015). Therefore, this research investigated the effect of micronisation of pre-soaked whole and dehulled bambara groundnut seeds on the nutritional, apparent viscosity, total extractable phenolics and antioxidant activity of their cooked samples in order to improve the utilisation of their resultant flours.

4.3.2 Materials and methods

4.3.2.1 Raw Materials

Mixed bambara groundnut seeds were obtained from Mbara produce market in Harare, Zimbabwe. The seeds were thoroughly cleaned to remove the chaff, shrivelled and broken seeds. The bambara groundnut seeds were then sorted based on the basis of seed size and colour of the eye. Cream bambara groundnut seeds with black eye colour were selected for this study. Uniform seed weight (77.54 g/100 seeds) and seed dimensions [(length 1.23 cm) × width (1.01 cm) × height (0.94 cm)] were used. The cleaned seeds were packed in air tight container and stored at 4 ^{o}C until time of use.

4.3.2.2 Methods

Hydrothermal process

The bambara groundnut seeds (100 g) were pre-soaked for 24 h at ambient temperature (25 ºC). Dehulling of pre-soaked bambara groundnut seeds was done manually by removing the seed coats after soaking. A tabletop microniser with 2 kW Phillips IR lamps (Technilamp Pty, Johannesburg, South Africa) was used for the current work. The microniser was first pre-heated for 20 min. The pre-soaked whole and dehulled samples were then micronised for 5, 10 and 15 min. After micronisation, the seeds were dried in a hot air dryer at 50 °C for 12 h. The samples were then cooled to room temperature for 1 h. The micronised seeds were milled to pass through a 500 µm aperture sieve using a Retsch ultra centrifugal mill (Model ZM200, Haan, Germany). The flours were stored in air tight container and kept at 4 °C until further analyses.

Preparation of soft porridge of flours from bambara groundnut samples

The resulting flours (2.5 g dry basis) of pre-soaked whole and dehulled bambara groundnut (unmicronised and micronised) samples in 25 mL of distilled water were cooked (10 min at 95 °C) into soft porridge (10% total solid), rapidly cooled down using Liquid Nitrogen and freeze dried. The freeze dried samples were stored in an air tight glass bottle and kept in the cold room at 4 °C for different analyses.

Preparation of extracts for total phenolic content and antioxidant capacity assays

Extracts from the cooked bambara groundnut (freeze dried) samples for total phenolic content and antioxidant capacity were prepared using acidified methanol (1% HCl in methanol) as described by Kayitesi (2013). 3 g of each sample was extracted with 30ml solvent in three phases as follows: 10 ml solvent was added to 3 g of the sample in a conical flask, stirred for 3h and centrifuged at 3500 *rpm* for 10 min at ambient temperature (25 ºC) and supernatant was decanted. The sample residue was rinsed again with 10 ml of the solvent stirred for 20 min centrifuged again as above and supernatant was decanted. This step was repeated. The supernatants were stored in an air tight glass bottle covered with aluminium foil and kept in the cold room at 4 °C until analysed.

4.3.2.3 **Analyses**

In-vitroProtein digestibility (IVPD)

A multi-enzyme method according to Hsu et al. (1977) and recently used by Vilakati et al. (2015) was used to determine the in-vitro protein digestibility of cooked bambara groundnut samples. Samples were digested with trypsin, 13,000- 20,000 BAEE units/mg protein (T03030, Sigma-Aldrich), bovine Chymotrypsin type II, 60 units/ mg protein (C4129, Sigma-Aldrich) and Protease XIV, 3.5 units/mg solid (P5747, Sigma-Aldrich) with pH adjusted (pH 8.0) with 0.1 M NaOH and incubated at 37 °C. The solution was maintained at this temperature and stirred continuously while the pH drop in the suspension was recorded over 10 min at 1 min interval. The percentage in vitro protein digestibility (IVPD) was calculated using the linear regression equation; Y = 210.46-18.10X (Hsu et al., 1977). Where Y is the percentage IVPD and X is the pH of sample suspension after 10 min hydrolysis.

In-vitro starch digestibility (IVSD)

In vitro starch digestibility of the cooked bambara groundnut samples was determined following the method described by Goni, et al. (1997) with some modifications. 50 mg starch of sample was used per assay and dispersed in 1 ml of boiling water. HCl-KCl buffer (10 ml) was added to each sample and the mixture was homogenised. Then 0.2 ml of solution containing 1 mg of pepsin (Sigma-Aldrich P7000-100G) was added to each sample and incubated at 40 ºC for 60 min in a shaking water bath. 10 ml of tris-maleate buffer was added and the pH was adjusted to 6.9 by using 1 M NaOH. The volume was then adjusted to 25 ml with tris-maleate buffer (pH 6.9) in a volumetric flask.

0.1 ml of aliquots was taken at 0 min and 5 ml tris-maleate buffer containing 2.6IU of α- amylase from porcine pancreas with the activity of 19.6 units/mg (Sigma-Aldrich A-3176) was added to the sample. The flasks were immediately placed in a shaking water bath at 37 ºC to start hydrolysis. 0.1 ml of aliquots were taken at 5 min and then after at 30 min interval until 3 h. Tubes containing the aliquots were placed in boiling water for 15minutes to inactivate α-amylase activity. Then, 1 ml of 0.4 M sodium acetate buffer (pH 4.75) and 200 µl of amyloglucosidase from *Aspergillus niger* with the activity of 64 U/ml (Megazyme E-AMGD-100 ml) were added and incubated for 45 min at 60 ºC to liberate the free glucose. Finally, the glucose concentration in the mixture was measured by using glucose oxidase peroxide.

The rate of hydrolysis was expressed as a percentage of the total starch hydrolysed at different times within 3 h. White wheat bread was used as a reference sample and was analysed within 20 min of baking. The following formula was used to calculate starch digestibility.

$$\text{Starch digestibility (\%)} = \frac{\text{mg starch digested}}{\text{mg starch in sample}} \times 100 \quad \ldots\ldots (1)$$

The starch classification based on its digestibility; the enzymatic hydrolyses method of Englyst et al. (1992) was used to determine rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS as the percentage of starch digested within 30min of incubation, SDS as the percentage of starch digested from 30- 120 min of incubation and RS was starch not digested after 120 min of incubation.

Estimated glycemic index

The hydrolysis curve (0-180 min) was fitted into a non-linear model established by Goni et al. (1997) and was used to describe the kinetics of starch hydrolysis:

$C = C\infty (1- e^{-kt})$…….. (2)

Where C is concentration at time t, C∞ is the percentage of starch hydrolysed after 180 min, K is the kinetic constant (min^{-1}) and t is the time (min). The parameters K and C∞ were estimated for each treatment based on the data which was obtained from the in vitro hydrolysis procedure. The hydrolysis curve (0-180 min) was fitted using OriginPro software (Exponential decay first order) to calculate the area under the hydrolysis curve (AUC).

The hydrolysis index (HI) as the area under the hydrolysis curve of treated sample divided by the corresponding area of white wheat bread. Then finally, the estimated glycemic index (EGI) was estimated by using the equation of Goni et al. (1997) as follows;

EGI = 39.71 + 0.549HI…….. (3)

Apparent viscosity analysis

The apparent viscosity of cooked soft porridge from pre-soaked unmicronised (0 min) and micronised bambara groundnut samples (5, 10 and 15 min) were determined according to method described by Muoki et al. (2013) with some modification. This was measured using the bob and cup accessory with a Physica MCR 101 Rheometer with Pheoplus software®, (Anton Paar, Ostfildern, Germany). The cooked soft porridge containing 10% solid (w/v) was prepared (95 ºC) and held at 50 ºC for 5 min to equilibrate and the cooked soft porridge was transferred into the rheometer set at 50 ºC. The measuring bob (diameter: 27 mm) was inserted into the cup (diameter: 28.9 mm) containing the cooked soft porridge. Solvent trap was used to prevent a moisture loss. The apparent viscosity was recorded at a shear rate of 0.01 to 1000 s^{-1}, the hysteresis area and power law parameters of the pastes were calculated using using OriginPro software.

The sample was stirred with a bob over a shear rate range of 0.01 to 1000 $s^{-1}$and the shear stress was determined. To describe the time independent flow behavior, the experimental data (shear stress- shear rate) were fitted by power law model;

$\tau = K \gamma^n$

Where, τ is shear stress (Pa), $\dot{\gamma}$ *is* the shear rate (s^{-1}), *K* is the consistency co-efficient ($Pa.s^n$) and n-value is the flow behavior index.

Determination of total phenolic content (TPC) of bambara groundnut

The total phenolic content of the extract samples were determined spectrophotometrically using the Folin–Ciocalteu procedure modified for 96-well plate as described by Apea-bah et al. (2014). Extract or catechin standard (18.2 µL) were dissolved in 1M HCl in methanol and pipetted into a 96-well microplate and 36.4 µL of 10% (v/v) Folin-Ciocalteu reagent in water added. Thereafter, 145.4 µL of 700 mM sodium carbonate was added and the plate incubated for 2 h in the dark at 25 ºC. Catechin standards of concentration 0.1 to 0.5 mg/mL prepared in 1M HCl in methanol was used for the calibration curve. The absorbance of the extracts and catechin standards were read at 750 nm using a microplate reader (Multiskan FC, Thermo Fisher Scientific, Shanghai, China) and the results expressed as mg catechin equivalents (CE)/g sample on dry weight basis.

Determination of antioxidant capacities of bambara groundnut

2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid ($ABTS^{+}$) assay was used to determine the antioxidant capacities (radical scavenging capacity) of the extracts. The ABTS assay is based on the generation of a blue/green $ABTS^{\bullet+}$ that can be reduced by antioxidants (Shelembe et al., 2012).The $ABTS^{\cdot+}$ radical scavenging activity of extract was determined following the procedure described by Awika et al. (2003). The reaction mixture consisting of 190 µL ABTS radical working solution generated by the addition of 58 mL phosphate buffer saline pH 7.4 to 2 mL ABTS mother solution (prepared by adding equal volumes of 3 mM potassium persulphate and 8 mM ABTS salt in water and left in the dark at room temperature for 16h) and 10µl extracts was incubated in the dark at ambient temperature (25 ºC) for 30 min in a 96-well plate. The absorbance was read at 750 nm. Trolox was used as the standard and results expressed as micromole Trolox equivalents per gram (µmol TE/g) sample on a dry weight basis.

Statistical analyses

The experimental work was repeated three times and the data was analysed using Statistica version 11 (StatSoftInc, Tulsa, USA). Multi-factor analysis of variance (MANOVA) was performed on all the measured values at $p \leq 0.05$. This was because the resulting flours of whole and dehulled bambara groundnut seeds after the treatment was in the same forms. Means were compared using the Fischer's least significant test (LSD) test at 5% level of significant. Independent variables were micronisation time (0, 5, 10 and 15 min) and dehulling treatment. Dependent variables were the measured values.

4.3.3 Result and discussion

4.3.3.1 In-vitro protein digestibility (IVPD)

Table 4.5 shows the effects of micronisation of pre-soaked micronised (whole and dehulled) bambara groundnut seeds on the *in vitro* protein digestibility of the cooked samples from their resulting flours. The *in vitro* protein digestibility in cooked samples from unmicronised whole and dehulled bambara groundnut samples were respectively 88.3 and 89.1%. Micronisation showed a non significant ($p>0.05$) increase in the *in vitro* protein digestibility of the cooked bambara groundnut whole and dehulled samples. The range of 88.6- 89.3% and 89.3- 90% were observed in the cooked samples from whole and dehulled samples respectively with micronisation for 5, 10 and 15 min.

The three different enzymes in multi-enzymes were used to estimates the hydrolysis of the protein in the stomach, small intestine and hind-gut of simple animals (Hsu et al., 1997). Micronisation reportedly has reduced nitrogen solubility index (NSI) suggesting protein of pre-soaked bambara groundnut (53%, 130 ºC) denatured (Ogundele et al., 2017) and this could enhance the availability of protein and increase its digestion by the digestive enzymes during wet heat processing. The in-vitro protein digestibilityof dehulled samples was slight higher than whole samples, which could suggest that removal of the seed coat from the pre-soaked bambara groundnut contributed to the increase in protein digestibility. Similarly, Vilakati et al. (2015) reported that micronisation caused a slight increase in protein digestibility of pre-conditioned dehulled cowpea (83.7%) to about 86.4 and 88.8%.

4.3.3.2 In-vitro kinetics of starch digestibility (IVSD)

Figure 4.12 shows the kinetic of starch digestion in cooked whole and dehulled bambara groundnut samples respectively. In general, as observed in all the cooked (unmicronised and micronised) bambara groundnut samples, rapid starch hydrolysis commenced in the first 5min and continueuntill it reached the maximum starch digestion at the hydrolysis time of about 90min.The amount of the starch digested in the cooked samples significantly ($p<0.001$) increased with hydrolysis time. The total starches digested at the 90 min (hydrolysis time) in cooked unmicronised whole and dehulled samples were 55% and 51% respectively. It was observed that

micronisation significantly ($p<0.001$) increased the starch digestion in the cooked bambara groundnut samples (whole and dehulled).

Table 4.5 Effects of micronisation of pre-soaked whole and dehulled bambara groundnut seeds on the protein digestibility of their cooked soft porridge from their resulting flours

Sample	Micronisation time (min)				
	0	5	10	15	Overall effect of dehulling
Whole	88.3^{a} (0.1)[1]	88.6^{b} (0.1)	89.1^{c} (0.1)	89.3^{cd} (0.1)	88.8^{a} (0.3)
Dehulled	89.1^{cd} (0.1)	89.3^{de} (0.1)	89.5^{e} (0.1)	90.0^{f} (0.2)	89.6^{b} (0.4)
Overall effect of micronisation time	88.7^{a} (0.4)	88.9^{b} (0.4)	89.3^{c} (0.2)	89.6^{d} (0.5)	

[1]*Means and Standard deviation n=3. Values with similar superscripts in rows are not significantly different ($p< 0.01$) Whole samples: Unmicronised 0 (0 min), Micronised: 5 (5 min), 10 (10 min), 15 (15 min). Dehulled samples: Unmicronised 0 (0 min), Micronised: 5 (5 min), 10 (10 min), 15 (15 min). Whole and dehulled samples were cooked and freeze dried resulting flours from pre-soaked micronised bambara groundnut seeds. Control (pre-soaked and unmicronised samples); treatment (pre-soaked and micronised samples at 5,10 & 15 min).*

The total starch digested in cooked whole micronised (5, 10 and 15 min) bambara groundnut samples at the 90min (hydrolysis time) ranged between 60 and 71%. Similarly, the total starch digested in cooked dehulled micronised (5, 10 and 15 min) bambara groundnut samples at the 90 min ranged between 67 and 71%.

The hydrolysis index (HI), estimated glycemic index (EGI) of cooked bambara groundnut (whole and dehulled) samples as influenced by micronisation are also shown in Table 4.6. Sandhu & Lim (2008) expressed hydrolysis index (HI) as the digestibility of the starch in foods as related to the digestibility of starch in a reference material (white bread). The hydrolysis indexes of cooked samples of unmicronised whole and dehulled bambara groundnut were 58 and 56% respectively. Micronisation significantly ($p<0.001$) increased the hydrolysis index (HI) in the cooked bambara groundnut samples. The hydrolysis index range of about 63- 75% was observed in cooked samples of whole micronised (5, 10 and 15 min) bambara groundnut. The hydrolysis index range of about 70- 77% was observed in the cooked samples of dehulled micronised bambara groundnut.

According to Kaur et al. (2010), the glycemic index of foods is greatly influenced by the starch digestibility in the food system. The estimatedglycemic indexes of cooked samples of unmicronised whole and dehulled bambara groundnut were 72 and 70% respectively. Micronisation also significantly ($p<0.001$) increased the estimated glycemic index (EGI) of cooked bambara groundnut samples. The estimated glycemic index range of about 75- 81% were observed in cooked whole micronised (5, 10 and 15 min) bambara groundnut samples and similar ranged of about 70- 80% were observed in cooked dehulled micronised (5, 10 and 15 min) bambara groundnut samples.

Table 4.6 also shows the effects of micronisation of pre-soaked (whole and dehulled) bambara groundnut seeds on the rapidly digesting starch (RDS), slowly digesting starch (SDS) and resistance starch (RS) contents of their cooked bambara groundnut samples. The RDS of 33 and 32%, SDS of 37 and 38% and RS of 29 and 24% were observed in cooked unmicronised whole and dehulledbambara groundnut respectively. Micronisation significantly ($p<0.001$) increased the RDS and SDS contents but significantly reduced the RS contents of cooked bambara groundnut (whole and dehulled) samples. The RDS and SDS represented a range of 37- 43.3% and 38.8- 46.7% of the starch digested in cooked samples of whole micronised bambara groundnut samples and 41-41.4% and 42.5- 44.6% in dehulled micronised bambara groundnut (5, 10 and 15min). The RS content of a range of 10-24% in cooked samples of micronised whole bambara groundnut and 14- 17% were observed in cooked samples of micronised dehulled bambara groundnut.

Micronisation has been reported to increase starch pre-gelatinisation (loss of crystalline nature of starch) in pre-soaked micronised bambaragroundnut (Ogundele et al., 2017) and with the further gelatinisation and solubilisation of the starch during cooking, the starch could become more accessible to the digestive enzymes. This could then be responsible for the increase in the starch digestibility of the cooked samples of micronised bambara groundnut samples. It has been reported that the gelatinisation of starch could facilitate the contact between substrate and digestive enzymes thus increasing the chances of hydrolysis (Alonso et al., 2000). The increase in digestible starch (RDS and SDS) and reduction in resistance starch RS could also be attributed to the increase in starch gelatinisation due to micronisation (Ogundele et al., 2017), causing more solubilisation of starch during cooking (polysaccharides such as amylose and amylopectin) and

becoming more digestible by the digestive enzymes. Hydrothermal treatment such as heat-moisture treatment (30% moisture, 120 ºC for 24 h) and autoclaving (121 ºC, 20 min) of pea, lentil and navy beans (Chung et al., 2010)) and mucuna beans (Siddhuraju & Becker, 2005) have been reported to increase digestible starch (RDS and SDS) and reduced resistance starch RS. Apart from the molecular change in starch (gelatinisation) responsible for the increased in the digestibility, other factor such as viscosity may influence the starch digestibility.High viscosity in cooked extrudate of cassava-soy porridge with added fine wheat bran was reported to be partly responsible for the low digestibility starch (Oladiran & Emmambux, 2016).

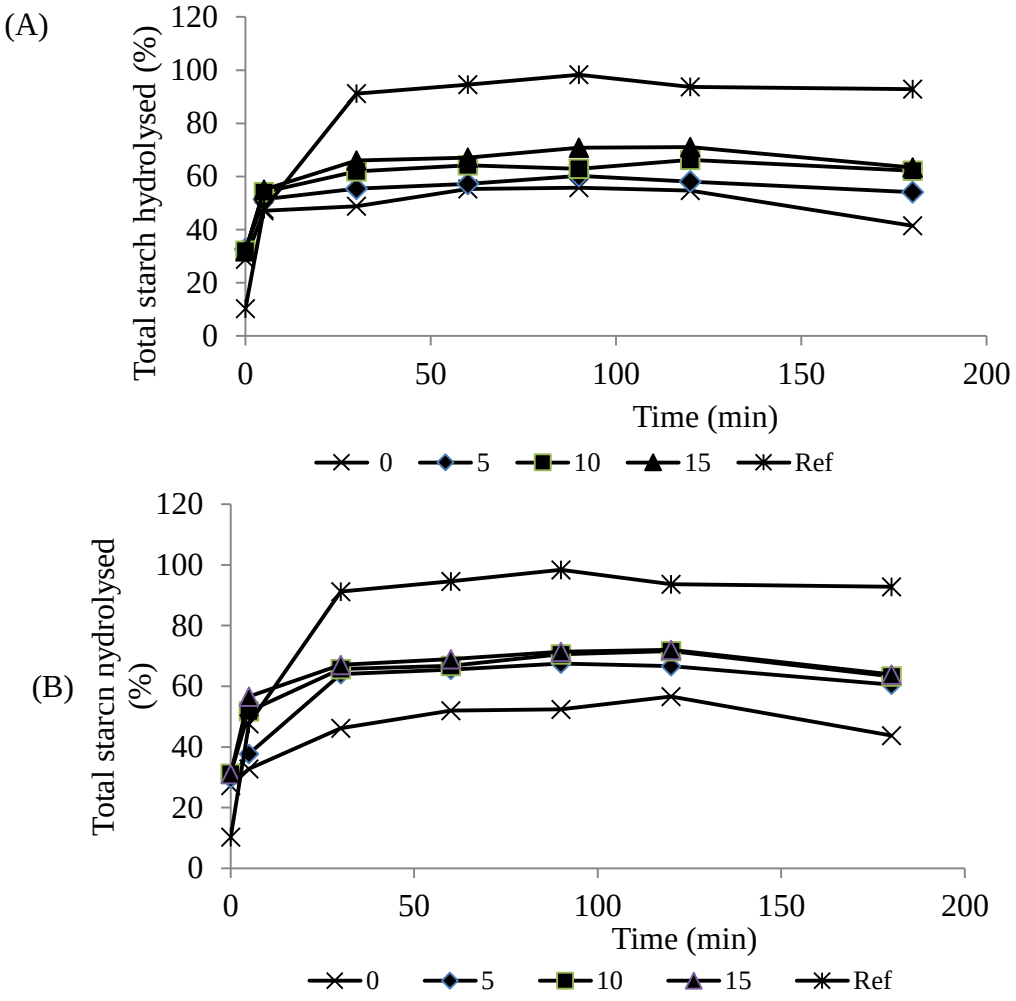


Figure 4.12 Effects of micronisation of whole (A) and dehulled (B) pre-soaked bambara groundnut seeds on the starch digestibility of cooked soft porridge from their resulting flours.
Whole samples: Unmicronised (0 min), Micronised: (5 min), (10 min), (15 min). Dehulled samples: Unmicronised (0min), Micronised: (5 min), (10 min), (15 min). Whole and dehulled samples were cooked and freeze dried soft porridge from resulting flour of bambara groundnut seeds. Ref (Reference sample): white wheat bread. Control (pre-soaked and unmicronised samples); treatments (pre-soaked and micronised samples at 5,10 & 15 min).

Table 4.6 Effects of micronisation of whole and dehulled pre-soaked bambara groundnut seeds on the starch digestibility of cooked soft porridge from their resulting flours.

Sample	Micronisation time (min)	C_∞ (%)	K ($\times 10^{-3} min^{-1}$)	HI (%)	EGI	RDS (%)	SDS (%)	RS (%)
Bread	Reference	98.3 (2.0)[1]	12.3 (0.0)	100.0 (3.1)	94.6 (1.7)	45.0 (0.4)	51.0 (0.5)	4.1 (0.0)
Whole	0	55.9^{a} (0.8)	5.0^{a} (0.0)	58.4^{b} (0.3)	71.8^{b} (0.1)	33.3^{b} (0.8)	37.8^{a} (0.4)	29.0^{e} (0.6)
	5	60.4^{c} (0.4)	6.9^{ab} (0.0)	63.4^{c} (0.7)	74.5^{c} (0.4)	37.0^{c} (0.2)	38.8^{b} (0.1)	24.2^{d} (0.2)
	10	66.2^{e} (0.2)	8.7^{ab} (0.0)	69.6^{d} (2.0)	77.9^{d} (1.1)	40.1^{d} (0.7)	42.8^{c} (0.1)	17.1^{c} (0.7)
	15	71.6^{g} (0.4)	9.8^{ab} (0.0)	74.9^{f} (0.4)	80.8^{f} (0.2)	43.3^{e} (0.5)	46.7^{e} (0.7)	10.0^{a} (0.5)
Dehulled	0	57.3^{b} (0.4)	5.0^{a} (0.0)	55.7^{a} (0.9)	70.2^{a} (0.5)	32.0^{a} (0.8)	38.6^{ab} (0.6)	30.0^{e} (0.3)
	5	68.0^{d} (0.9)	9.3^{ab} (0.0)	70.1^{d} (1.0)	78.2^{d} (0.6)	41.0^{de} (0.4)	42.5^{c} (0.6)	16.6^{c} (0.3)
	10	73.2^{f} (0.0)	9.8^{ab} (0.0)	75.3^{ef} (1.1)	81.0^{ef} (0.6)	41.0^{de} (0.3)	44.3^{d} (0.9)	15.1^{b} (1.3)
	15	75.4^{h} (0.6)	11.3^{c} (0.0)	77.3^{f} (0.2)	82.2^{f} (0.1)	41.4^{de} (1.0)	44.6^{d} (0.4)	14.1^{b} (0.8)

[1]*Means and Standard deviation n=3. Values with different superscripts in columns are significantly different (p< 0.01).*
Whole samples: Unmicronised (0 min), Micronised: (5 min), (10 min), (15 min). Dehulled samples: Unmicronised (0min),Micronised: (5 min), (10 min), (15 min). Whole and dehulled samples were cooked and freeze dried soft porridge from resulting flour of bambara groundnut seeds. Ref (Reference sample): white wheat bread. Control (pre-soaked and unmicronised samples); treatments (pre-soaked and micronised samples at 5,10 & 15 min).
C_∞, percentage of starch hydrolysed after 180min; k, kinetic constant (min^{-1}); HI, hydrolysis index; EGI, estimated glycemic index.RDS, readily digestible starch; SDS, slowly digestible starch; RS, resistant starch.

4.3.3.3 **Apparent viscosity**

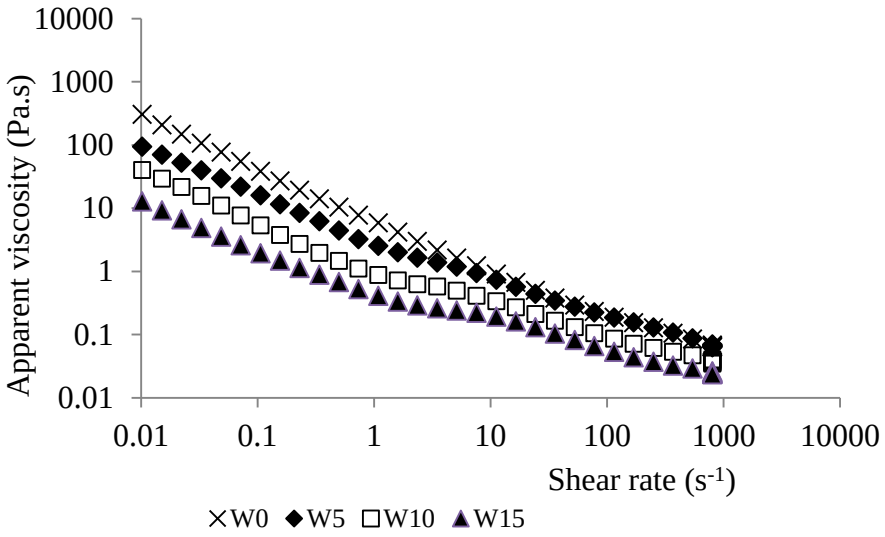
Figure 4.13 shows the apparent viscosity of cooked samples of whole (A) and dehulled(B) bambara groundnut samples at 10% solid contents (50ºC). Micronisation (5, 10 and 15min) significantly ($p<0.0001$) reduced the apparent viscosity of cooked samples bambara groundnut samples at the shear rates (Zero shear and $100s^{-1}$) compared to unmicronised (Table 4.7). At 10% solid contents for the cooked samples, the consistency index (*K-values*) for both unmicronised whole and dehulled samples were 2.64 and 2.76 respectively (Table 4.7). Micronisation significantly ($p<0.001$) reduced the consistency index for both cooked whole and dehulled bambara groundnut samples. The consistency indexes in cooked whole bambara groundnut samples were reduced by 24, 66 and 85% with micronisation (5, 10 and 15min). Similarly, in dehulled samples the consistency indexes were reduced by 28, 70 and 73% with micronisation. The n-values were significantly ($p>0.001$) different from each other with micronisation (Table 4.7) and shows the cooked samples of bambara groundnut (unmicronised and micronised) exhibited a non-linear behavior ($n<1$) and were shear thinning. The range of n-values of the cooked samples (10% solid contents, 50ºC) of whole and dehulled bambara groundnut were between 0.45- 0.52. The hysteresis area loop of the cooked samples of both whole and dehulled samples showed a significant ($p< 0.001$) reduction with micronisation (Table 4.7).

In previous chapter of this research, micronisation reportedly reduced pasting viscosity of resulting flour from pre-soaked bambara groundnut due to microstructural changes of starch in protein matrices. It was then expected that the starch and protein interaction as reported which was partly responsible for the reduction in the viscosities of micronisedbambara groundnut samples may enhance the decrease in the starch digestibility. On the contrary, it was observed that the decrease in apparent viscosities may suggest there is more interactions between substrates and digestive enzymes hence the increase in starch digestion.

The results in this current study show that the decrease in the viscosity of cooked soft bambara groundnut porridge may be related to the increase in the starch digestibility with micronisation and wet heat cooking. The viscosity in cooked extrudate of cassava-soy porridge with added fine wheat bran partly contributed to the increase in the enzymes digestible starch (Oladiran & Emmambux, 2016).This could be due to gelatinisation and possibly depolymerisation of starch which may occur during the cooking process thereby making the starch more readily accessible

for enzymatic hydrolysis. As observed in chapter 2 of this research, starch pre-gelatinisation increased in pre-soaked bambara groundnut with micronisation. Gelatinisation of starch facilitates the interaction of substrate and digestive enzymes therefore increasing the chances of hydrolysis (Alonso et al., 2000).

(A)



(B)

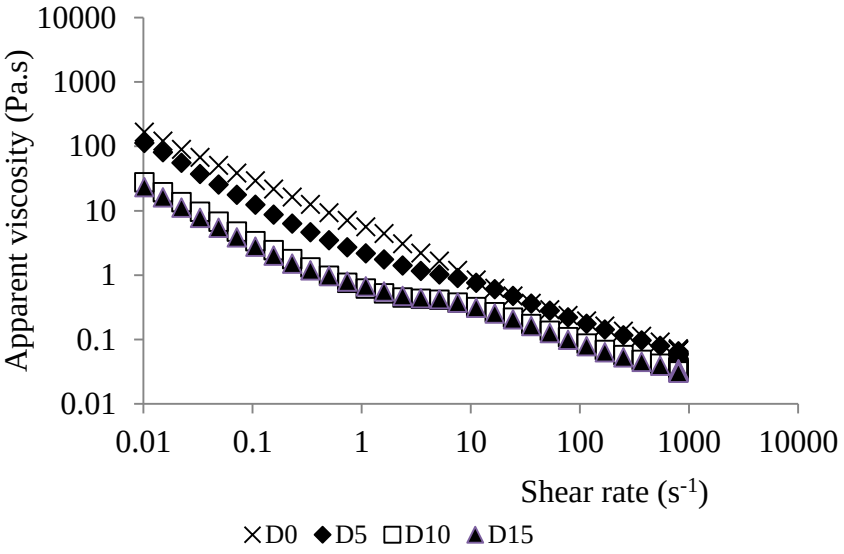


Figure 4.13 Effects of micronisation of whole (A) and dehulled (B) pre-soaked bambara groundnut seeds on the apparent viscosity of their cooked soft porridge at 50ºC at different shear rates.

Apparent viscosity of soft porridge from resulting flour of whole bambara groundnut samples: 0 min (unmicronised), Micronised: 5 min , 10 min and 15 min. Apparent viscosity of soft porridge from resulting flours of dehulled bambara groundnut samples: 0 min (unmicronised), micronised : 5 min, 10 min and 15 min. Control (pre-soaked and unmicronised samples); treatments (pre-soaked and micronised samples at 5,10 & 15 min).

Table 4.7 Effects of micronisation of pre-soaked whole and dehulled bambara groundnut seeds on the apparent viscosity, power law parameters (K and n) and Hysteresis of cooked soft porridge (10% solids) from their resulting flours at 50 ºC.

Samples	Micronisation time (min)	Viscosity (mPa.s)		K-value (Pa.s)^n	n-value	Hysteresis area (Pa/s)
		Zero shear viscosity	Viscosity at 100s^{-1}shear rate			
Whole	0	557.25^{g} (34.51)	0.17^{cd} (0.02)	2.64^{d} (0.20)[1]	0.43^{a} (0.01)	6392.30^{g} (70.15)
	5	417.25^{e} (43.51)	0.17^{cd} (0.02)	2.00^{c} (0.10)	0.47^{c} (0.00)	5223.04^{e} (223.22)
	10	157.75^{c} (19.32)	0.08^{b} (0.01)	0.90^{b} (0.04)	0.50^{d} (0.01)	3458.29^{d} (317.45)
	15	46.38^{a} (3.730	0.05^{a} (0.01)	0.40^{a} (0.06)	0.52^{e} (0.01)	1628.12^{a} (21.96)
Dehulled	0	467.50^{f} (26.41)	0.18^{d} (0.02)	2.76^{d} (0.06)	0.42^{a} (0.00)	6606.84^{g} (265.00)
	5	460.50^{f} (15.80)	0.15^{c} (0.01)	2.00^{c} (0.09)	0.45^{b} (0.00)	5775.51^{f} (0.00)
	10	234.75^{d} (13.84)	0.08^{b} (0.01)	0.80^{b} (0.05)	0.50^{d} (0.01)	3014.11^{c} (29.69)
	15	120.50^{b} (10.41)	0.06^{ab} (0.01)	0.76^{b} (0.05)	0.50^{d} (0.00)	2454.01^{b} (29.69)

[1]*Means and Standard deviation n=3. Values with different superscripts in columns are significantly different ($p<0.05$).*
Zero shear viscosity (mPa.s) and Viscosity at 100 s^{-1} shearrate (mPa.s) of soft porridge from resulting flours of whole bambara groundnut samples: 0 min (unmicronised), Micronised: 5 min , 10 min and 15 min. Zero shear viscosity (mPa.s) and Viscosity at 100 s^{-1} shearrate (mPa.s) of soft porridge from resulting flours of dehulled bambara groundnut samples: 0 min (unmicronised), Micronised: 5 min , 10 min and 15 min. Control (pre-soaked and unmicronised samples); treatment (pre-soaked and micronised samples at 5,10 & 15 min).
Power law parameters of soft porridge from resulting flours of whole bambara groundnut samples: 0min (unmicronised), Micronised: 5min , 10min and 15min. Power law parameters of soft porridge from resulting flours of dehulled bambara groundnut samples: 0min (unmicronised), micronised : 5min, 10min and 15min .
K-value, consistency index or the consistency coefficient (Pa.s)^n, n-value, flow behaviour index and Hysteresia area (Pa/s).

4.3.3.4 **Total phenolic compound and Antioxidant properties**

The total phenolic content and the radical scavenging activities of extracts of cooked bambara groundnut samples are shown in Table 4.8.The total phenolic contents of the extract of cooked unmicronised whole bambara groundnut sample was 2.52 mg CE/g dry basis and micronisation significantly ($p<0.001$) increase the total phenolic content of extract of cooked whole bambara groundnut samples. Similarly, It appeared that the $ABTS^+$ radical scavenging activities of extracts of cooked whole bambara groundnut samples also significantly ($p<0.001$) increased with micronisation (5, 10 and 15 min). The opposite was the case for the extracts of cooked dehulled bambara groundnut samples. Total phenolic content of extract of cooked unmicronised dehulled bambara groundnut samples (1.76 mg CE/g) significantly ($p<0.001$) decrease with micronisation. In addition, the $ABTS^+$ radical scavenging activities of extracts of cooked dehulled bambara groundnut samples significantly ($p<0.001$) decrease with micronisation. The total phenolic content and $ABTS^+$ radical scavenging activitiesof extracts of cooked whole bambara groundnut samples are significantly ($p<0.001$) higher than the cooked dehulled bambara groundnut samples.

The increase obtained in the results of the total phenol contents and antioxidant activities ($ABTS^+$) of the extracts of cooked samples of whole micronised bambara groundnut may be attributed to the changes in cell wall components of whole micronised bambara groundnut, solubilisation of cell wall components such as pectin or presence of other bioactive compounds in the whole bambara groundnut. As observed in the previous chapter (Section 4.1), the solubilisation of pectin in micronised bambara groundnut was responsible for the microstuctural change in the cotylendon such as cell wall separation and distruption of the parenchyma cells. The breakdown of cell structures during heat processing of green beans was reported may lead to the release of phenolic compounds linked to various cell wall structures (Jiratanan & Liu, 2004). This suggested that upon cooking some phenolics could become more extractable and also there could be a possible release of bound phenolic forms.

Conversely, the decrease of the total phenol contents and antioxidant activities ($ABTS^+$) of the extracts of cooked samples of dehulled micronised bambara groundnut may be attributed to the absence of the seed coat.

Table 4.8 Effects of micronisation of whole and dehulled pre-soaked bambara groundnut seeds on the total phenolic compound and antioxidant property of extracts of cooked soft porridge from their resulting flours

	Whole samples				Dehulled samples			
	Micronisation time (min)				Micronisation time (min)			
	0	5	10	15	0	5	10	15
TPC*	2.54^{d} (0.04)[1]	2.88^{e}(0.03)	3.04^{e} (0.13)	3.84^{f} (0.51)	1.76^{c} (0.02)	1.45^{bc} (0.03)	1.31^{ab} (0.02)	1.03^{a} (0.02)
Antioxidant properties**								
$ABTS^{+}$	35.66^{c} (1.33)	39.68^{d} (0.96)	43.63^{e} (0.66)	46.07^{f} (0.91)	31.14^{b} (1.70)	29.60^{b} (0.45)	25.97^{a} (2.51)	24.66^{a} (1.37)

[1]*Means and Standard deviation n=3. Values with different superscripts in rows are significantly different (p< 0.001).*
Whole samples: Unmicronised (0 min), Micronised: (5 min), (10 min), (15 min). Dehulled samples: Unmicronised (0 min), Micronised: (5 min), (10 min), (15 min). Whole and dehulled samples were extracts from cooked and freeze dried soft porridge from resulting flour of bambara groundnut seeds. Control (pre-soaked and unmicronised samples); treatment (pre-soaked and micronised samples at 5,10 & 15 min).
*TPC, Total phenolic compound; Antioxidant property: $ABTS^{\cdot+}$radical scavenging capacity. *Expressed as mg catechin equivalents mg (CE)/g sample on dry weight basis.** Expressed as micromole Trolox equivalents per gram (µmol TE/g) sample on a dry weight basis*

Luthria & Pastor-Corrales, (2006) reported that phenolic compounds upon heating may undergo degradation and oxidation. This decrease suggest that upon cooking of the dehulled micronised bambara groundnut samples, some phenolics were rendered less extractable, due to the interaction of the phenolic compound and other bioactive compounds with other food components such as proteins (Bishnoi et al., 1994). The complexation of seeds phenolic compound with its macromolecules such as proteins have been reported may reduce its availability and extractability (Awika et al., 2003).

Phenolic compound is majorly deposited in the seed coat of legume seeds. This could be responsible for the high total phenolic content and antioxidant activities in the whole samples than dehulled samples. The removing the seeds coat of pre-soaked bambara groundnut seeds could be responsible for the reduction in the availability of the phenolic compound and its antioxidant activity potentials.

Thermally heating legume whole seeds prior to the flours such as marama (Kayitesi et al., 2010) and yellow soybeans (Xu & Chang 2008a) reportedly increased the total phenolic contents and antioxidant activities compared to the untreated. However, Kayitesi (2013) attributed the loss in the total phenolic content and antioxidant activities in flour from cooked micronised pre-conditioned whole cowpea seeds (41%, 153 ºC) possibly to the leaching out of the phenolic compound into the water during the cooking. A high concentration of total phenolic contents with corresponding high antioxidant activities has been reported in seed coats of thermally treated black soybean (Xu et al., 2008b) and pinto and navy bean (Anton et al., 2008) than its cotyledon.

4.3.4 Conclusions

Micronisation increases starch digestibility and estimated glycemic index of cooked bambara groundnut soft porridge samples. This is as a result of changes in rheological properties of cooked bambara groundnut soft porridge samples during wet heat cooking. Micronisation and wet heat cooking increases the antioxidant property of whole bambara groundnut soft porridge. Dehulling of bambara groundnut prior to micronisation reduces the antioxidant property. Resulting flour of micronised pre-soaked bambara groundnut produces a soft porridge, thus,

increase protein and energy density which possess compounds capable of scavenging free radicals.

4.3.5 References

Adebowale, Y. A., Schwarzenbolz, U., & Henle, T., 2011. Protein isolates from bambara groundnut *(Voandzeia Subterranean L.)*: Chemical characterization and functional properties. *International Journal of Food Properties*, 14, 758-775.

Alhassan, G., Kalu, B., & Egbe, O., 2012. Influence of planting densities on the performance of intercropped bambara groundnut with cowpea in Makurdi, Benue state, Nigeria. *International Journal of Development and Sustainability*, 1, 860-879.

Alonso, R., Aguirre, A., & Marzo, F., 2000. Effects of extrusion and traditional processing methods on anti-oxidant and *in-vitro* digestibility of protein and starch in faba and kidney beans.*Food Chemistry*, 68,159-165.

Alozie, Y. E., Iyam, M. A., Lawal, O., Udofia, U., & Ani, I. F., 2009. Utilization of bambara groundnut flour blends in bread production. *Journal of Food Technology*, 7, 111-114.

Andrejko, D., Rydzak, L., Slaska-Grzywna, B., Goÿdziewska, M., & Kobus, Z., 2008. Influence of preliminary thermal processing applying infra-red radiation on pea seeds cooking process. *International Agrophysics*, 22, 17-20.

Anton, A. A., Ross, K. A., Beta, T., Fulcher, R. G., & Arntfield, S. D., 2008. Effect of pre-dehulling treatments on some nutritional and physical properties of navy and pinto beans *(Phaseolus vulgaris L.). LWT-Food Science and Technology*, 41, 771-778.

Apea-Bah, F. B., Minnaar, A., Bester, M. J., & Duodu, K. G., 2014. Does a sorghum–cowpea composite porridge hold promise for contributing to alleviating oxidative stress? *Food Chemistry*, 157, 157-166.

Arntfield, S., Scanlon, M., Malcolmson, L., Watts, B., Cenkowski, S., Ryland, D., & Savoie, V., 2001. Reduction in lentil cooking time using micronization: comparison of 2 micronization temperatures. *Journal of Food Science*, 66, 500-505.

Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., & Cisneros-Zevallos, L., 2003.Screening methods to measure antioxidant activity of sorghum *(Sorghum bicolor)* and sorghum products.*Journal of Agricultural and Food Chemistry*, 51, 6657-6662.

Azam-Ali, S., Sesay, A., Karikari, S., Massawe, F., Aguilar-Manjarrez, J., Bannayan, M., & Hampson, K., 2001. Assessing the potential of an underutilized crop–a case study using bambara groundnut. *Experimental Agriculture*, 37, 433-472.

Bishnoi, S., Khetarpaul, N., & Yadav, R.K., 1994. Effect of domestic processing and cooking methods on phytic acid and polyphenol contents of pea cultivars *(Pisum sativum)*. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*, 45, 381–388.

Chung, H.J., Liu, Q., & Hoover, R., 2010.Effect of single and dual hydrothermal treatments on the crystalline structure, thermal properties, and nutritional fractions of pea, lentil, and navy bean starches. *Food Research International*, 43, 501-508.

Eltayeb, A. R. S., Ali, A. O., Abou-Arab, A. A., & Abu-Salem, F. M., 2011. Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut *(Vigna subterranean)*. *African Journal of Food Science*, 5, 82-90.

Englyst, H.N., Kingman, S.M., & Cummings, J.H., 1992. Classification and measurement of nutritionally important starch fractions.*European Journal of Clinical Nutrition* 46: Suppl. 2, S33–S50.

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F.A., 1997. Starch hydrolysis procedure to estimate glycemic index. *Nutrition Research*,17:427-437.

Hillocks, R., Bennett, C., & Mponda, O., 2012. Bambara nut: A review of utilisation, market potential and crop improvement. *African Crop Science Journal*, 20.

Hsu, H., Vavak, D., Satterlee, L., & Miller, G., 1977. A multi-enzyme technique for estimating protein digestibility. *Journal of Food Science*, 42, 1269-1273.

Jideani, V. A., & Diedericks, C. F., 2014. Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*.*Antioxidant-Antidiabetic Agents and Human Health*, 187.

Jiratanan, T., & Liu, R. H., 2004. Antioxidant activity of processed table beets *(Beta vulgaris var, conditiva)* and green beans *(Phaseolus vulgaris L.)*. *Journal of Agricultural and Food Chemistry*, 52, 2659-2670.

Kaur, M., Sandhu, K. S., & Lim, S. T., 2010. Microstructure, physicochemical properties and *in vitro* digestibility of starches from different Indian lentil *(Lens culinaris)* cultivars.*Carbohydrate Polymers*, 79, 349-355.

Kayitesi, E., 2013. Micronisation of cowpeas: Effects on sensory quality, phenolic compounds and bioactive properties.University of Pretoria, Pretoria.

Kayitesi, E., Duodu, K. G., Minnaar, A., & De Kock, H. L., 2012. Effect of micronisation of pre-conditioned cowpeas on cooking time and sensory properties of cooked cowpeas. *Journal of the Science of Food and Agriculture*, 93, 838-845.

Luthria, D.L., & Pastor-Corrales, M.A., 2006. Phenolic acids content of fifteen dry edible bean *(Phaseolus vulgaris L.)* varieties. *Journal of Food Composition and Analysis*, 19, 205–211.

Lyimo, J. M. M., 2000. Evaluation of the nutritional quality and acceptability of sorghum-based tempe as potential weaning foods in Tanzania. *International Journal of Food Sciences and Nutrition*, 51, 269-277.

Muoki P.N., De kock H.L., & Emmambux N.M., 2014.Effect of soy flour addition and heat processing method on nutritional quality and consumer acceptability of cassava complementary porridges. *Journal of the Science of Food and Agriculture*, 92, 1771-1779.

Nithiyanantham, S., Selvakumar, S., & Siddhuraju, P., 2012. Total phenolic content and antioxidant activity of two different solvent extracts from raw and processed legumes, *Cicer arietinum L*. and *Pisum sativum L*. *Journal of Food Composition and Analysis*, 27, 52-60.

Nnam, N. 2001. Chemical, sensory and rheological properties of porridges from processed sorghum *(Sorghum bicolor)*, bambara groundnut *(Vigna subterranea L. Verdc)* and sweet potato *(Ipomoea batatas)* flours. *Plant Foods for Human Nutrition*, 56, 251-264.

Nyau, V., Prakash, S., Rodrigues, J., & Farrant, J., 2015. Antioxidant activities of bambara groundnuts as assessed by FRAP and DPPH assays. *American Journal of Food and Nutrition*, 3, 7-11.

Ogundele, O. M., Minnaar, A., & Emmambux, M. N., 2017. Effects of micronisation and dehulling of pre-soaked bambara groundnut seeds on microstructure and functionality of the resulting flours. *Food Chemistry*, 214, 655-663.

Oladiran, D. A., & Emmambux, N. M., 2016. Effects of extrusion cooking and wheat bran substitution on the functional, nutritional, and rheological properties of cassava-defatted toasted soy composite. *Starch-Stärke*, 68, 1–9.

Onimawo, I., Momoh, A., & Usman, A., 1998. Proximate composition and functional properties of four cultivars of bambara groundnut *(Voandezeia subterranea). Plant Foods for Human Nutrition*, 53, 153-158.

Ouedraogo, M., Ouedraogo, J. T., Tignere, J. B., Bilma, D., Dabire, C. B., & Konate, G., 2008. Characterization and evaluation of accessions of Bambara groundnut *(Vigna subterranea (L.)Verdcourt)* from Burkina Faso. *Sciences & Nature*, 5, 191-197.

Rehman, Z.U., & Shah, W., 2005. Thermal heat processing effects on anti-nutrients, protein and starch digestibility of food legumes. *Food Chemistry*, 91, 327-331.

Sandhu, K. S., & Lim, S.T., 2008. Digestibility of legume starches as influenced by their physical and structural properties. *Carbohydrate polymers*, 71, 245-252.

Siddhuraju, P., & Becker, K., 2001. Effect of various domestic processing methods on antinutrients and in vitro protein and starch digestibility of two indigenous varieties of Indian tribal pulse, *Mucuna pruriens var. utilis*.*Journal of Agricultural and Food Chemistry*, 49, 3058-3067.

Shelembe, J. S., Cromarty, D., Bester, M. J., Minnaar, A., & Duodu, K. G., 2012. Characterisation of phenolic acids, flavonoids, pro-anthocyanidins and antioxidant activity of water extracts from seed coats of marama bean *(Tylosema esculentum).* 47(3), 648-655.

Siddhuraju, P., & Becker, K., 2005. Nutritional and antinutritional composition, *in vitro* amino acid availability, starch digestibility and predicted glycemic index of differentially processed mucuna beans *(Mucuna pruriens var. utilis)*: an under-utilised legume. *Food Chemistry*, 91, 275-286.

Vilakati, N., Macintyre, U., Oelofse, A., & Taylor, J. R., 2015. Influence of micronization (infrared treatment) on the protein and functional quality of a ready-to-eat sorghum-cowpea African porridge for young child-feeding. *LWT-Food Science and Technology*, 63, 1191-1198.

Xu, B., & Chang, S. K., 2008a.Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. *Journal of Agricultural and Food Chemistry*, 56, 7165-7175.

Xu, B., & Chang, S. K., 2008b. Antioxidant capacity of seed coat, dehulled bean, and whole black soybeans in relation to their distributions of total phenolics, phenolic acids, anthocyanins, and isoflavones.*Journal of Agricultural and Food Chemistry*, 56b, 8365-8373.

5 GENERAL DISCUSSION

The general discussion provides a critical review on some of the methodologies used in this study. Furthermore, the effect of micronisation of pre-soaked bambara groundnut on cooking characteristics of the seeds, functional properties with a model to explain the role of micronisation of pre-soaked bambara groundnut in inducing the low pasting viscosity of resulting flours, also, nutritional and health promoting properties of the resulting flours will be discussed. Finally, a look at the future research, including the application of flour from micronised bambara groundnut seeds in a food system is discussed.

5.1 Methodology: Critical review

5.1.1 Selection of raw material

The general conclusions drawn about the effects of micronisation of bambara groundnuts in terms of cooking quality and the functional and nutritional and health benefits of resulting flours in this research are based only on the cream black eye bambara groundnut seeds. Only one type of bambara groundnut was used due to availability of the cream black eye bambara groundnut seeds. However the results can still be comparable to other coloured-type of bambara groundnut seeds because the cotyledon of bambara groundnut seeds can be considered similar despite the differences in there seed coat colours (Chibarabada et al., 2014). Kayitesi et al. (2012) reported no significant difference in the cooking time of unmicronised pre-conditioned light bechuana white cowpeas and dark Glenda cowpeas.

5.1.2 Soaking and dehulling pre-treament

In this study, soaking was used as a moisture pre-conditioning treatment prior to micronisation instead of the tempering as used in most studies of micronisation of other legumes (Kayitesi et al., 2012; Khattab&Arntfield, 2009; Bellido et al., 2006; Mwangwela et al., 2006). Bambara groundnuts pre-soaked in this study was able to achieve the 53% moisture level in short time (24h) than the tempering that achieve up to 50% moisture in 48 h. Soaking of cowpea seeds was reported to cause changes in the size of starch granules (Phadi, 2004); this may have also contributed to the changes in the cooking characteristics of the bambara groundnut seeds. For the dehulled samples, pre-soaked whole bambara groundnut seeds were manually dehulled by hand. The pre-soaking of the seeds facilitates the manual removal of seed coat. However, manual

dehulling procedure in this study was tedious, resulted in split of most of the seeds, and this method may not be representation of a commercial dehulling/seed coat removal process of grains for example tangential abrasive dehulling device (TADD).

TADD is a grain dehuller which loosens seed coat of pre-conditioned grain and facilitated separation from the cotyledon thus reducing losses (Opoku et al., 2003). However, several attempts made using TADD dehuller with or without pre-conditioned bambara groundnut seeds led to breaking of seeds, scratching of the cotyledon surface, elevated loss and partially dehulledseeds as earlier reported with cowpeas (Penicela, 2011). The process was tedious to operate with bambara groundnut seeds probably because it was design for harder kernel grains such as sorghum (Bean et al., 2006). The structural damages on dehulled cowpea seed as results of using TADD dehuller affected the cooking characteristics of the dehulled cowpea seeds (Penicela, 2011). For example the scratched on the cotyledon surfaces of dehulled seeds could influence its rate of water absorption during soaking and cooking thus influencing cooking time as well as its sensory attribute like texture (Penicela, 2011).

5.1.3 Micronisation

Micronisation is an application of far infrared radiation with a pre-soaked bambara groundnut seeds. This infrared radiation (non-ionising radiation) falls within wavelength of about 3 to 1000 µm (Krishnamurthy et al., 2008). A table-top Microniser (FIR) with three tubular quartz infrared lamps (2 kW per lamp) and a thermocouple thermometer for monitoring temperature changes were used for this study.Microniser is an effective heating process due to its uniformity in heating; short processing time and energy saving (Krishnamurthy et al. 2008). However, the laboratory scale microniser was design for small quantity of samples. The microniser was manually operated at 130 ºC surface temperature (25 cm away from the infrared energy source) and pre-soaked bambara groundnut seed samples were stationary. The samples were turned half way of each time interval to ensure uniform or homogenous heat distribution in seeds as reported by Mwangwela et al. (2006) on smaller seeds such as cowpea.

Micronisation (FIR) is a surface heat treatment because of its low penetrating power (Krishnamurthy et al., 2008). It is not certain if the results from this study with the manual laboratory table top microniser could be relatively similar to the commercial scale microniser.

Wiriyaumpaiwong et al. (2004) reported high colour change and urease inactivation of pre-conditioned soybean micronised using industrial scale microniser than laboratory scale. This was due to the possible difference in their operational conditions such as feed rates, capacity, energy source and vibrating units. Therefore, further studies should be done to investigate the effect of manual laboratory table top microniser compared to a commercial scale microniser on the results of this study. It is also not certain if the manual laboratory table topmicroniser can be upscale but the results from this study suggest relative differences to their control (pre-soaked unmicronisedbambara groundnut samples).

5.1.4 Drying

After micronisation, the entire sample had high residual moisture. The end moisture of pre-soaked micronised bambara groundnut seed for 5, 10 and 15 min were about 40, 30 and 20% respectively. Micronisation of tempered varieties of cowpeas (small seeds size) seeds at 153 ºC reduced the moisture content from 41% to about 8-13% (Mwangwela et al, 2006). Drying grains enhanced other processes such as milling into flours (Newkirk, 2010) and ensuring shelf stability during storage of grains or products because of low water activity (Slade et al., 1991). The oven drying of seeds (50 ºC) was used to reduce the moisture of the micronised pre-soaked bambara groundnut (seeds) samples to a moisture level of about 7- 12% in this study. The temperature of drying (50 ºC) in this study was high enough only to evaporate the moisture, but potentially not causing any chemical changes on starch and protein especially in the unmicronisedbambara ground samples. Starch gelatinisation of bambara groundnut occurred at about 68-76 °C (Kaptso et al., 2015; Sirivongpaisal, 2008) and protein denaturation occurred at about 68- 80 °C (Kaptso et al., 2015; Kudre et al., 2013). It is assumed that the drying at 50 ºC did not cause any molecular changes and this need to be confirmed in further studies. Thus the difference in data to show effects of micronisation is relative rather than absolute values.

5.1.5 Water absorption

The water absorption was determined in this study using the rapid screen method described by Waniska& Myers, (2006). It was used to measure the amount of water absorbed by the bambara groundnut seed samples over the soaking (24 h) and cooking (150 min) incubation time. The rapid screen method of water absorption minimised the amount of sample needed for the analysis (Waniska& Myers, 2006). However, the challenge of water absorption of the seeds in this study

was that the blot drying of the seeds with absorbent paper was difficult. There could be possibility that the seeds may either be over or under blotted before the weight of seeds is taken but necessary precaution was taken to ensure relative comparison of the results to the control samples.

5.1.6 Cooking time

Proctor and Watts (1987) described cooking time as the time necessary for cowpea seeds to attain a soft cooked texture acceptable to consumers. Mattson cooker has been mostly used by various authors to determine cooking time of legume seeds (Kayitesi et al., 2012; Mwangwela et al., 2006; Wang et al., 2003; Abdul-Kadiret al., 1990). A Mattson bean cooker with light rods (49.8g each) used in this study show the difference in cooking time of pre-soaked micronised bambara groundnuts to the unmicronised as reported by Kayitesi et al. (2012) with cowpea.The cooking time of sample was determined at the point when 20 out of the 25 pins (80%) dropped through the cooked seeds as the bean softened. Certain factors such as the weight of the rods (Wang et al., 2003) and number of rods (Abdul-Kadiret al., 1990) contributed to the differences in cooking time of legume seeds.The light rods (49.8 g each) suggested better prediction of beans cooking time than heavy weight rods (90g each) (Proctor & Watts, 1987) and the heavy weight rods underestimate the cooking time (Mwangwela, 2006).

The limitations observed during the use of Mattson bean cooker for this study are as follows;

(i) The bambara groundnut especially with whole seeds characterised with a smooth surface (Chibarabada et al., 2014), makes it slippery for the pins to stand on the seeds and positioning of the cooker into the boiling water difficult.

(ii) The cooking process is an open system process and the pins were manually placed on the beans which make it a tedious procedure.

(iii) With open system moisture loss as steam into the surrounding during cooking of the seeds which may actually result in an underestimation of the cooking time. The level of the boiling water in the open system in this study was however kept throughout the cooking by topping with boiled water to mininise the moisture loss. A closed transparent cover could improve the efficiency of the manual Mattson cooker cooking time prediction. The use of an automated system (Mattson cooker) will address most of these limitations, relief the operator and will be most effective methods for predicting the cooking time (Wang &Daun, 2005), however, it is expensive.

5.1.7 Microscopy

A representative samples was ensured in the cotyledon of the seeds by ensuring that samples were taken at the centre part of the cotyledon all the time. The advantages and limitations of different microscopy techniques used in this study are presented in Table 5.1. Light microscopy with simple setup in sample preparation provided details on the structural changes on starch. However, its limitations include poor resolution images and possible introduction of artefacts (Autio&Salmenkallio-Marttila, 2001). Compared to light microscopy, the resolution of electron microscopy is significantly higher and it generates three dimensional images (Aguilera and Stanley, 1999). SEM provided surface details of the cotyledon that cannot be otherwise resolved with light microscopy. However, material thickness can obscure important cellular details. With the CLSM, high resolution three dimensional images are possible (Dürrenberger et al., 2001). The CLSM provided details on protein, the protein matrix was adequately differentiated based on the colour (red colour) as the Nile red bind with protein to fluorescence (Brooke, 1991; Heertje et al., 1987). The black colour assumed to embed starch granules is not certain however the shape and size seem to be like starch granules.

5.1.8 Rapid Visco Analyser

Rapid Visco Analyser (RVA) is a tool for evaluating the physica**l** attributes of various viscousmaterials during controlled cycles of heating and cooling (Crosbie& Ross, 2007). Rapid Visco Analyser (RVA) was used in this study to determine the swelling and stability of starch and other components such as protein in resulting flours from unmicronised and micronised pre-soaked bambara groundnut in a pasting curve as reported by Mwangwela et al. (2007a) on resulting flour from pre-conditioned cowpea (41% moisture, 130 and 170 ºC). It was made clear that the presences of starch alone did not result in low pasting viscosity (Mwangwela et al. (2007a), other components such as protein and pectin in the resulting flour contributed to the results obtained. The preparation of sample is very important in obtaining consistent and accurate results on Rapid Visco Analyser (RVA) (Crosbie & Ross, 2007). Therefore, in this study, water was first measured into the canister before flour sample was added and suspension was manually stirred using the paddle prior to being placed in RVA in order to prevent lumping. The formation of lumps in suspension could result in variability among repetitions because the RVA measures the resistance to flow, thus a well dispersed fluid is important to prevent noise in the viscosity measurement.

Table 5.1 Advantages and limitations of different microscopy techniques

Microscopy[1]	Application[1]	Sample[1]	Significant steps in preparation[1]	Advantages [2,3,4]	Limitations[2,3,4]
Bright-field (Normal light)	Viewed stained samples: parenchyma cells, starch granules, cell wall material (pectin).	Seed/Flour	Seed: Fixation, Dehydration, Polymerization, Sectioning (1µm) and Staining (Toluidene blue) Flour: Dispersion (30% glycerol) Staining (iodine)	Simple setup with very little preparation required for samples	Gives blur image if sample too thick, Low contrast with little natural pigmentation, so samples usually need staining. Staining may destroy or introduce artefacts
Polarised-light	Viewed unstained samples: starch granules (loss of birefringence).	Flour	Dispersion in glycerol (30%) without staining (iodine)	Simple setup provides contrast to unstained cells for clear observation	Strong illumination may damage delicate samples
SEM	Viewed the surface details of samples: seed cotyledon parenchyma.	Seed	Cryo-fixation (Liquid nitrogen) facilitated sample cracking, fractions (10 mm) mounted on aluminium stubs and Coated with gold (20nm thick)	Revealed surface details of cotyledon that cannot be otherwise resolved with light microscopy techniques. Images have a greater resolving power than light	Samples need to be coated in a conductive material before viewed in the SEM. The thickness of the material can obscure important cellular details
CLSM (Fluorescence)	Viewed starch protein interaction (aggregates) in High resolution images)	Flour	Staining (Safranin O dye)	Not restricted by thickness, less time and less labourious sample preparation, not likely change the structure of samples	Samples needs staining (fluorochrome) to be visible, staining can produce artefacts. Flurochromes sensitivity to laser illumination and can bleach within necessary time for searching and acquiring images

[1] In the current study, [2]Bandyopadhyay et al., 2013; [3]Dürrenberger et al., 2001; [4]Autio &Salmenkallio-Marttila, 2001; [5]Mckenna, 1997.

RVA has the ability to measure viscosity property of macromolecules such as starch and protein of samples but with limitation of measuring other rheological properties such as visco-elastic properties (Goode et al., 2005).

5.1.9 Nitrogen solubility index

Nitrogen solubility index in this study was used to determine the changes in protein of resulting flour from unmicronised and micronised pre-soaked (53% moisture, 130 °C) bambara groundnut seeds. The reduction in nitrogen solubility of legumes flour upon micronisation of the pre-conditioned seeds suggested denaturation of protein as loss of solubility to aggregation due to exposure of hydrophobic amino acids (Mwangwela et al., 2007b; Zheng et al., 1998). The bambara groundnut resulting flour samples were centrifuged twice with added NaCl in order to extract all the solubilised proteins. Legumes major storage protein is globulins and they are soluble in salt solution (Kiosseoglou & Paraskevopoulou, 2011). In the preliminary study variation were indicated in the results of the liquid extract possibly because the soluble protein were diluted and this was below detection limit of the Dumatherm. Therefore, the filtrates were freeze dried to concentrate the soluble protein to improve the detection level of the Dumatherm and ensured the repeatability of the results.

5.1.10 Nutritional properties

5.1.10.1 Protein digestibility

Theprotein digestibility was determined in this study using enzymatic methods as described by Hsu et al. (1977) and also used by Vilakati et al. (2015). This method was used to determine the protein digestion of the soft cooked porridge of bambara groundnut samples. The *in vitro* multi-enzyme technique for determination of protein digestibility was used because it closely mimics protein digestion in humans (stomach and small intestine) as it uses pancreatic porcine intestinal peptidases, trypsin and chymotrypsin as used in the method (Vilakati et al., 2015; Hsu et al., 1977). The proteases hydrolyse of the proteins breaking peptide bonds, to produce smaller peptides and amino acids (Bond &Beynon, 1987). The multi-enzyme technique required short time, high degree sensitivity and effective in predicting apparent digestible protein of food samples and products (Hsu et al., 1977). Factors such as sample characteristics, enzyme activity (concentration, temperature, pH and composition) and digestion time among others influences

the *in vitro* digestibility assays (Hur et al., 2011). The results of *in vitro* were different to the *in vivo* digestion because of the difficulties in accurately mimicking the highly complex physicochemical and physiological event occurring in animal and human tracts (Hur et al., 2011).

Hamaker et al. (1986) *invitro* protein digestibility method was used with cereal grains such as maize and sorghum known with poor protein quality. Prolamins are the major storage protein in sorghum with deficiency in the essential amino acid lysine (Taylor et al., 2006) and it is located within starchy endosperm of this grain (Duodu et al., 2003). The *in vitro* pepsin protein digestibility uses only pepsin enzymes and pepsin only estimates the protein hydrolysis in the stomach (Boisen&Eggum, 1991). However, legume with good protein quality contains globulins with rich lysine as the major storage protein (Kiosseoglou&Paraskevopoulou, 2011). In contrary to *in vitro* pepsin method, Hsu et al. (1977) multi enzyme method estimates hydrolysis in the stomach, small intestine and hind gut of simple stomach of animals. Therefore, more bonds will be hydrolysed by the multi-enzyme than the pepsin method. The multi-enzymes method indicated that during proteolysis protons are released from cleaved peptide bonds thus resulting in a decrease in pH in a suspension (Boisen&Eggum, 1991). The *in vitro* protein digestibility obtained with the pH-stat method (keeping the pH constant with continuous addition of NaOH and measuring NaOH consumption) has been correlated with true protein digestibility values determined in rats (Boisen & Eggum, 1991). Compared with the pH-drop method, the pH-stat method improved the prediction of protein digestibility (Boisen&Eggum, 1991).

5.1.10.2 **Starch digestibility**

Thestarch digestibility was determined in this study using the *in vitro* method described by Goni et al. (1997). This method was used to determine the starch hydrolysis to predict the glycaemic index of cooked soft porridge of bambara groundnut samples. The combination of enzymes closely simulates starch digestion in the small intestine where most of starch digestion takes place (Hasjimet al., 2010). In the *in vitro starch* digestibility, porcine pepsin enzyme hydrolyzed the protein; α-amylase hydrolyzed α-1, 4-glycosidic bonds in the starch molecules into oligosaccharides and dextrins; and the amyloglucosidase (AMG) hydrolyzed the α-1, 6-glycosidic bonds in dextrinsto glucose (Goni et al., 1997).

Goniet al. (1997) and Englystet al. (1996) have closely correlated the *in vitro* method to an *in vivo*method. However, the use of Goniet al. (1997) method may be limited based on some related food and human physiological factors affecting starch digestion such as gastric emptying rate, digest a viscosity and transit time in the gastrointestinal tract (Turnbull et al., 2005). Other factors such as use of mechanical methods such as grinding, homogenisation and milling (Woolnoughet al., 2008) and chewing which may introduced salivary α-amylase at the oral stage (Bornhorst& Singh, 2012) may negatively affect precision of the assay but was avoidable considering the nature of the samples. In this study however, the oral digestion phase was excluded since soft porridge, which was the food sample under study, is usually swallowed without mastication. Soft porridge has a short residence time in the mouth and therefore the effect of oral digestion on starch digestion in the porridge was assumed negligible. The type of mixing that occurs at the oral phase can sufficiently be provided by a shaking water bath (Woolnough et al., 2008).

5.1.11 Apparent Viscosity

The apparent viscosity of the cooked soft porridge samples was determined using a rheometer at varying shear rates of 0.01 s^{-1} – 1000 s^{-1}. Bob and cup method was used as a measuring system, a measuring bob inserted into a cup containing sample. The soft porridge was transferred cautiously into the cup in order to avoid formation of air bubble which may affect estimation of the viscosity. Rheometer measures viscosity as the resistance of fluid to flow. The presence of bubbles in suspensions causes reduction in velocity gradient near the bubbles and thus there is less viscosity than where there are no bubbles (Rust & Manga, 2002). A layer of paraffin oil was spread on top of the sample to avoid moisture loss. Samples were allowed to equilibrate to ensure uniform temperature in samples before the test was conducted. It is important to note that Rheometer can perform the function of RVA, both methods in this study measure viscosity of cooked soft porridge samples as a function of shear and temperatures. However, Rheometer which operates under controlled stress and shear rate mode of operation can provide other rheological properties such as shear thinning and viscoelastic properties of samples (Tabilo-Munizaga& Barbosa-Cánovas, 2005). In this study, the soft porridge of bambara groundnut is shear thinning material, where the viscosity decreases with increase in shear rate. However, presence and concentration of solutes such as protein and lipids may also influence rheological behavior of pastes (Yoo &Yoo, 2005).

5.1.12 Bioactive and antioxidant properties

Acidified methanol was used to extract the free extractable dietary phenolics from the freeze dried samples of soft cooked bambara groundnut porridge. The choice of 1% (v/v) HCl-methanol was based on its suitability for extracting bioactive compounds (free phenolic contents) such as anthocyanins, phenolic acids and flavonoids (Dykes et al., 2005; Awika& Rooney, 2004). Acidification of extraction solvents enhanced phenolic extraction probably by promoting plant cell wall disintegration and solubilisation of the component phenolic compounds (Shelembeet al., 2014; Chirinos et al., 2007). It is worth to note that extracts of the porridge may contain other various food components such as peptides, oligosaccharides, reducing sugars and lipids. Hence, the assay (phenolic content and antioxidant activities) may suffer interference with non-phenolic reducing substances such as ascorbic acid, reducing sugars and many nitrogenous compounds (Ainsworth & Gillespie, 2007), therefore, making it rather challenging in attributing the antioxidant capacities solely to the concentration of the constituent dietary phenolics (Nderitu et al., 2013). However, the total phenolic content of the porridge was used to indicate the contribution of the phenolic compounds to their radical scavenging activities.

The FolinCiocalteu assay was used as a simple, rapid and inexpensive method to determine the total phenolic content of the samples (Dykes & Rooney, 2006; Awika& Rooney, 2004). It is very sensitive and widely used for studying phenolic antioxidants (Awika& Rooney, 2004). The assay is based on the electron transfer mechanism. In this assay, the analyte (phenolic compound) reacts with the Folin Ciocalteu reagent, under basic conditions, through dissociation of the phenolic hydroxyl group leading to formation of a phenolate anion (MacDonald-Wicks et al., 2006). This then reduced the yellow acidic Folin Ciocalteu reagent (containing phosphomolybdate-phosphotungstate ions) to blue molybdenum-tungsten complex with absorption maxima at 765 nm (Prior et al., 2005). However, other analytes with reducing properties such as ascorbic acid, reducing sugars and many nitrogenous compounds may also test positive to this assay (Ainsworth & Gillespie, 2007). Therefore, this may result in over-estimation of the total phenolic content of the plant-based samples since they contain such compounds with reducing properties. It is therefore suggested that the FolinCiocalteu reaction should be considered as a measure of the total reducing capacity (Prior et al., 2005) or total antioxidant capacity (Everetteet al., 2010) of the samples. Furthermore, since phenolic

compounds are the most abundant antioxidants in most plants, the Folin Ciocalteu assay gives a good approximation of the total phenolic content of plant-based samples (Everette et al., 2010).

The total antioxidant activity of bambara groundnut extracts was determined using the ABTS assays. The ABTS assay measures the ability of antioxidants present in a sample to scavenge the ABTS radical cation ($ABTS^{\bullet+}$) as compared to Trolox (a water soluble vitamin E analogue) which results in loss of the bluish-green colour of the radical (Re et al., 1999). The ABTS assay is ideal to determine the antioxidant capacity of both hydrophilic and lipophilic compounds in samples since the radical is soluble in both aqueous and organic solvents (Arnao et al., 2001). The limitation of ABTS assay is that it is not biologically relevant assay since ABTS radical cation are not found in the human body and the values obtained are thus of little physiological relevance (Prior et al., 2005). However the assay is useful for determining trends in radical scavenging activities of different samples.

5.2 Research findings and future work

In this research, cooking characteristics of pre-soaked micronised bambara groundnut seeds, functional properties of the resultant flour and nutritional and bioactive properties of cooked soft porridge are summarised in Figures 5.1, 5.2 and 5.3.

Therefore, in this section a thesis will be put forward on the effect of changes of seed physicochemical properties and microstructure such as starch, protein and pectin towards the reduction in cooking time in micronised pre-soaked (53% moisture, 130 °C) seeds. The thesis will also put forward on how the changes in seed physicochemical of starch and protein contributed towards related functional properties of resulting flour and nutritional and bioactive properties of cooked soft porridge. Lastly, potential future work on applications of flour from micronised pre-soaked micronised bambara groundnut seeds in food systems will be explored.

5.2.1 Cooking characteristics

A schematic diagram explaining the effect of micronisation of pre-soaked (53% moisture, 130 °C) bambara groundnut seeds on physicochemical properties and seed microstructure as related to the cooking characteristics was presented in Figure 5.1. It demonstrated that micronisation of the pre-soaked bambara groundnut seed samples caused an increase in solubilisation of pectin as shown in Table 4.1.2.

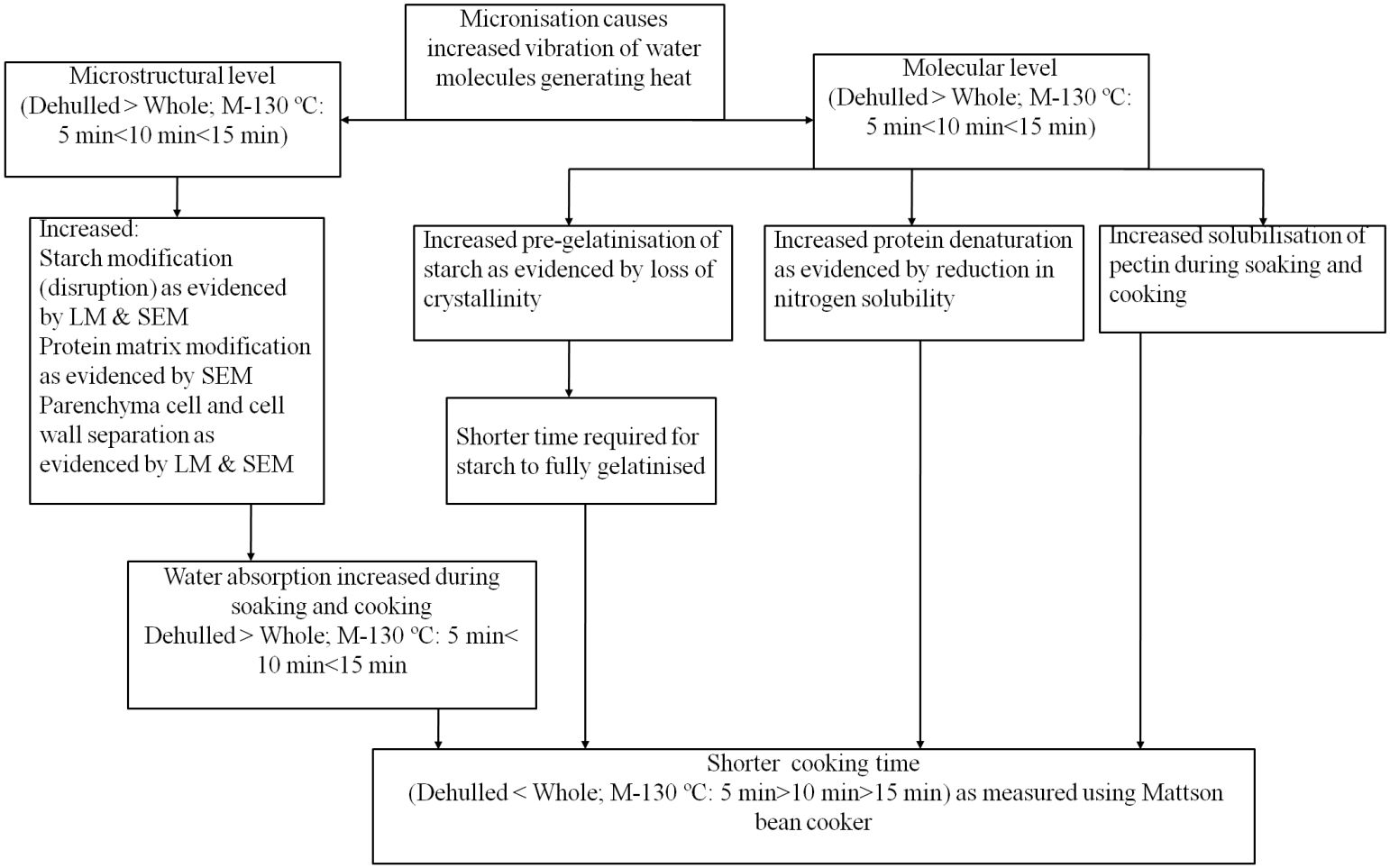


Figure 5.1Schematic representation of the effects of micronisation of pre-soaked (53% moisture, 130 °C) on bambara groundnut physicochemical properties and seed microstructure as related to cooking characteristics of bambara groundnut seeds

> = High; < = Less.*Whole samples: Unmicronised (0 min), Micronised: (5 min), (10 min), (15 min). Dehulled samples: Unmicronised (0 min),Micronised: (5 min), (10 min), (15 min)*

Pectin solubilisation is one of important physicochemical changes in legume seeds during cooking for softening to take place (Bernal et al., 1997). The degradation of pectin in the middle lamella reduced pectin to lower molecular weight products through the beta elimination reaction and it indicated separation of parenchyma cells (Liu et al., 1993b). It is clear that the solubilisation of the pectin has a role to play for the cell separation in the pre-soaked micronised bambara groundnut seed samples compared to unmicronised as shown in Figures 4.2 and 4.3. Pectin solubilisation was responsible for the rapid hydration of the micronised bambara groundnut seeds, released of turgor pressure of the parenchyma cellsdue to cell wall became weak and collapsed (mechanical stress) (Gonçalves et al., 2007; Greve et al., 1994) and softening; thus shorter cooking time as compared to unmicronised during cooking as previously reported by Ndungu et al. (2012) on micronisation of moisture-conditioned seeds of cowpea. The increased solubility of pectin in pre-soaked micronised bambara groundnut seeds confirmed previous reports in moisture-conditioned and micronised lentils (Arntfieldet al., 2001) and cowpea (Ndungu et al., 2012). In addition to pectin solubilisation, denatured protein network in micronised seeds (130 °C) contributed to reduction in cooking time by assisting in holding water for starch granules in order to swell and gelatinise faster during cooking (Mwangwela, 2006).

The reduction in cooking time of pre-soaked whole bambara groundnut seeds micronised (130 ºC) for 15 min as shown in table 4.1 was higher than what other previous authors have reported in moisture- conditioned micronised small seeds (Kayitesi et al., 2012; Mwangwela et al., 2006). The reason for this difference could be related to the level of moisture used prior to micronisation of the seeds and differences in seed size. Small seeded legume had more changes in physicochemical properties than bigger seeds due to more energy penetration (Fasina et al., 2001). Water is a dipolar based on its dipole moment which in turns resulted in having electronegative oxygen and electropositive hydrogen atoms (Michael et al., 1991). In the relation of importance of water to infrared heating process, the interaction of water with microwave heating is similar to infrared radiation because both converts electromagnetic waves to heat. The dielectric property of microwave heating shows water possessed the ability to store energy (large dielectric constant) due to the hydrogen bond and the energy stored can be converted as heat (Venkatesh&Raghavan, 2004; Grant & Halstead, 1998). In IR radiation, for a molecule like water to absorb the energy the vibrations must cause a net change in the dipole moment of the molecule and the molecular vibrations cause intermolecular friction among molecules and these

results in generation of heat (Sharma, 2009). The 53% moisture level therefore suggest that the presence of the water in bambara groundnut seeds caused increased in energy absorption, molecular vibration and intermolecular friction of water molecules and other biomolecules (starch, protein and pectin). Hence, resulting in more heat generation and water evaporation than in others reported micronisation studies with 40% moisture or lower.

Micronisation time is a critical parameter responsible for the extent of the microstructural changes in pre-soaked bambara groundnut seeds. The extent of disruption of starch granules, disintegration of middle lamella and cell separation in pre-soaked micronised bambara groundnut seeds were dependent on the increase in the micronisation time. In agreement with Mwangwela et al. (2006), extent of cell separation facilitated rapid water absorption and softening of pre-conditioned micronised cowpea seeds during cooking. The increase in water absorption during cooking of pre-soaked bambara groundnut seeds was similar to the results of Mwangwela et al. (2006) and Andrejko et al. (2008) on pre-conditioned micronised cowpea and pea respectively. The higher water absorption in these pre-conditioned micronised legume seeds compared to unmicronised was related to reduction in their time of cooking. In this study, increase in extent of cell separation in pre-soaked bambara groundnut seeds with micronisation indicated more rapid water absorption during cooking thus softening of the seeds would occur in shorter time compared to the other previously reported.

5.2.2 Functional properties

Micronisation caused starch pre-gelatinisation (Figure 4.8) and reduction in nitrogen solubility index as evidence of protein denaturation (Table 4.4) in micronised pre-soaked bambara groundnut. This was similar to the results of previous authors on pre-conditioned legumes (Mwangwela et al., 2007a; Bellido et al., 2006; Arntfield et al., 2001). The high moisture and increase in micronisation time in this study caused an increase in the extent of molecular changes in biomolecules such starch and protein. The extent of these molecular changes on these biomolecules such as starch, and protein could be more than the previous studies reported. This clearly affected the measured functional properties such as swelling, water solubility and pasting viscosity of the resulting flours as also reported by previous authors on functionality of resulting flour from moisture conditioned micronised cowpea seeds (Vilakati et al., 2015; Mwangwela et al., 2007b). Water solubility index of flour for example is highly dependent on the protein

solubility (Damodaran, 1996). The increase in surface hydrophobicity of the denatured proteins of micronised seeds suggests lowering protein solubility by unfolding of the protein molecules and exposure of buried hydrophobic sites (Zheng et al., 1998; Mwangwela et al., 2007a).

Table 5.2 Summary of viscosity, percentage nitrogen solubility and aggregation of cooked soft porridge from resulting flours from bambara groundnut (whole and dehulled) at 50 ºC

Soft cooked Porridge	Micronisation (min)	Viscosity (mPa.s)			NSI (%)	Estimated Aggregates	
		Zero shear viscosity	Viscosity at 100 s^{-1}	RVA		size (µm)	Number
Whole	0	↓ 557	↓ 0.19	↓ 347	↓ 134	-	-
	5	417	0.18	204	132	↑ 146	↑ 1
	10	157	0.08	133	76	167	4
	15	46	0.05	48	42	170	13
Dehulled	0	↓ 467	↓ 0.20	↓ 456	↓ 142	-	-
	5	460	0.15	165	138	↑ 123	↑ 1
	10	234	0.08	74	90	137	6
	15	120	0.06	52	42	200	18

↓= Decrease, ↑= Increase, - = No aggregates formed

The resulting flour from pre-soaked micronised bambara groundnut seeds had a reduction in the pasting viscosity (Table 5.2). Mwangwela et al. (2007b) reported a similar reduction in pasting viscosity of resulting flour from micronised pre-conditioned cowpea seeds at different surface temperatures (130-170 ºC). However, the mechanism responsible for this phenomenon was not proposed. Schematic diagram proposing the role of the aggregates (starch-protein matrices) in the low viscosity of the resulting flours during cooking is presented (Figure 5.2). In this research the mechanism was proposed with some evidences of partial starch gelatinisation, protein denaturation and most importantly aggregation of starch granules embedded in protein matrices

with possible exposed hydrophobic sites in resulting flour of micronised pre-soaked bambara groundnut (Figure 5.2).

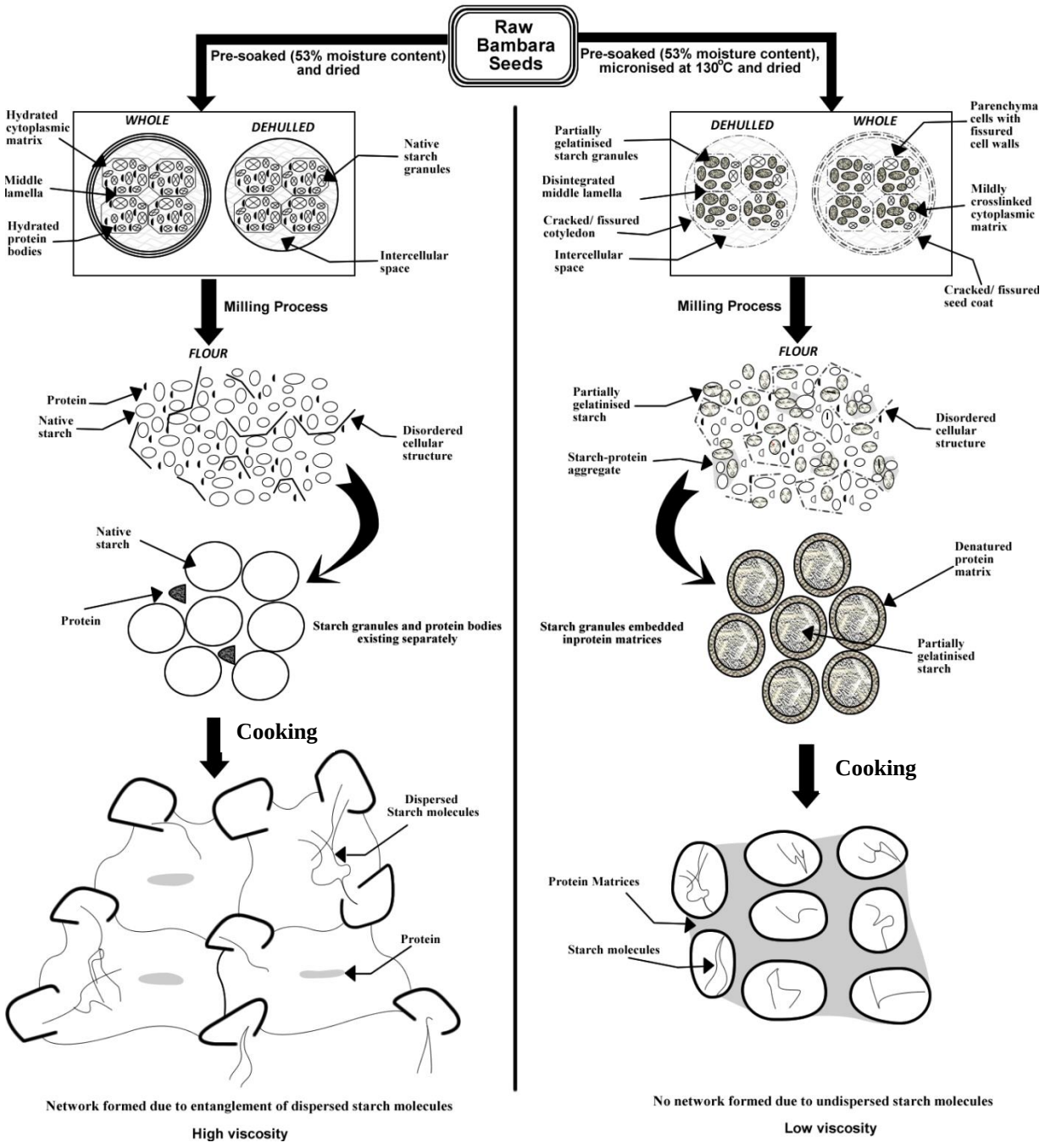
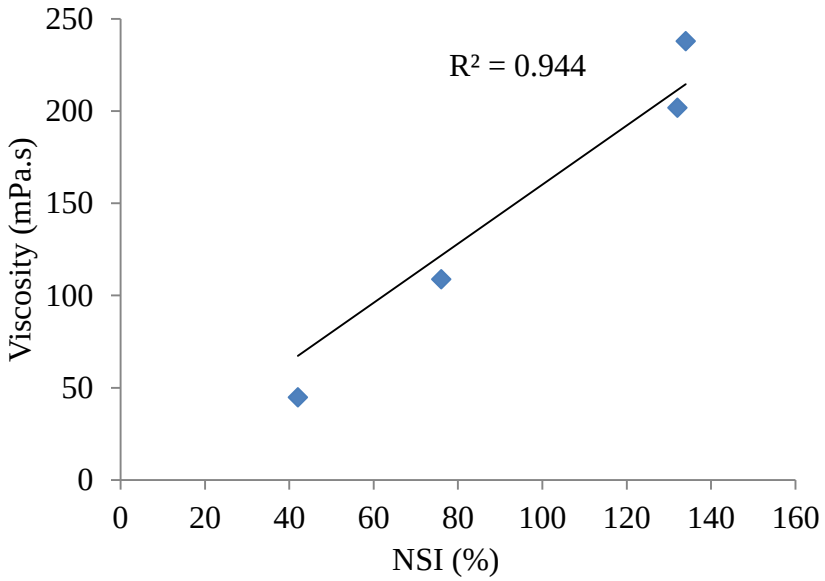


Figure 5.2 Schematic representation of probable role of aggregates of starch- protein matrices in low viscosity of resulting flour from micronised (53% moisture, 130 °C) bambara groundnut during cooking

(A)



(B)

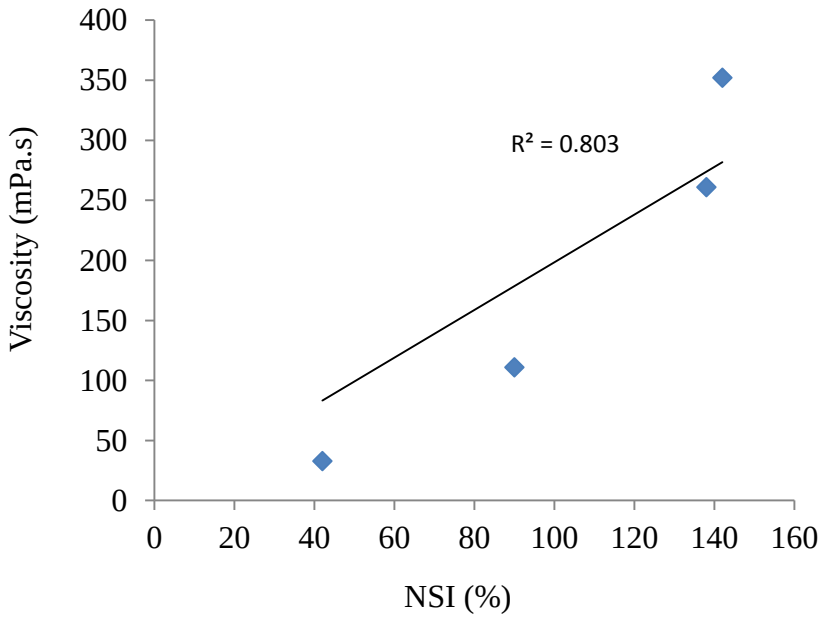


Figure 5.2 Correlation of NSI and viscosity of cooked soft whole (A) and dehulled (B) samples bambara groundnut porridge.

A positive correlation for the whole (A) and dehulled (B) sample was 0.944 and 0.803 respectively.

It is proposed that during cooking, starch granules from unmicronised pre-soaked bambara groundnut swells, breaks and the starch molecules such as amylose and amylopectin will be dispersed causing an increase in the viscosity of the suspension. During cooling, there is further increase in the viscosity due to formation of junction zones through hydrogen bond interaction between starch molecules to form entanglements which have more resistance to flow (High viscosity). However, in micronised samples, starch granules may not swell because of the protein coating surrounding the starch granules which is hydrophobic in nature. When starch molecules are not allowed to leach out and be dispersed in micronised sample suspension, the starch molecules would not form a junction and this result in decrease in viscosity. This implies that the aggregates in the micronised samples porridge would not disperse and would thus remain intact. This probably caused reduction in hydrodynamic volume of the starch molecules, hence contributing to the low pasting viscosity as observed inmicronised samples. Correlation analysis was done to show the relationship between the NSI and viscosity of the cooked soft bambara groundnut porridge (Figure 5.3). A strong positive correlation of 0.944 and 0.803 was found between the NSI and viscosity in both whole and dehulled samples respectively with micronisation. The relationship indicated that as the NSI decreased, the viscosity decreased as well. This suggests the role of the thermally denatured protein due to micronisation on the viscosity of the cooked soft bambara groundnut porridge.

5.2.3 Nutritional

Cooked soft porridge of micronised samples markedly had improved nutritional quality. Energy and protein quality are useful to access the adequacy of starch and protein in foods and effect of processing on their adequacy to tackle the protein energy malnutrition especially in infant. Protein quality in cooked micronised soft porridge of bambara groundnut samples were improved in terms of their digestibility as compared to the unmicronised samples (Table 4.5) as reported by Vilakati et al. (2015) on porridge of pre-conditioned micronised dehulled cowpea. With denaturation of protein in cooked micronised porridge, the protein digestibility would increase due to the increasing availability of the protein into lower fractions. This implies that there is adequacy of protein to meet the protein needs for infant and children if considered as complementary food ingredients (Vilakati et al., 2015).

The digestion of starch in cooked micronised soft bambara groundnut porridge (30 min) of about 0.1 to 0.83 was higher as compared to 0.03 to 0.09 of unmicronised samples (Figure 4.12). This suggested that the digestion of the gelatinised starch in the cooked micronised porridge was faster compared to unmicronised samples. In addition, micronisation and wet heat cooking increased the estimated glycemic index of cooked micronised soft bambaragroundnut porridge (Table 5.3). The EGI of about 75- 80% suggests the cooked micronised soft bambara groundnut porridge is a high energy dense food. The increase in solubilisation of starch may increase its enzyme digestibility (Alonso et al., 2000), possibly increasing the estimated glycemic index of cooked extruded porridge (Muoki et al., 2012). Porridge with high energy density is desirable energy source for feeding infant because the food facilitates weight gain (Brand- Miller et al., 2002).

The cooked micronised porridge of bambara groundnut samples were less viscous compared to unmicronised cooked porridge at zero and 100 s^{-1} shear rate (Table 5.2). These shear rates resemble the maximum shear developed in human mouth during mastication of porridge between 0.1 to 10 mPa.s viscosity (Mezger, 2006). At zero shear rate, the viscosity values at different micronisation of whole and dehulled samples (50 ºC) correspond with the viscosity values measured with the RVA method (Table 5.2). The viscosity of about 0.05 - 0.18 mPa.s was found in micronised cooked porridge (100 s^{-1} shear rate) at 10% solid content. Several authors identified 1- 3 mPa.s viscosity of complementary porridge as suitable for infant feeding (Muoki et al., 2015; Muoki et al., 2012; Treche & Mborne, 1999). The reduction in viscosity in this study shows a relationship with the increase in starch digestibility of cooked micronised bambara groundnut porridge samples. It is suggests that the viscosity of the resulting flour from micronised bambara groundnut can be useful as complementary ingredients with other food ingredients such as cereal and tubers, which are known with high viscosity and energy density (Muoki et al., 2015; Peroni et al., 2006; Chen &Ramaswamy, 1999).

5.2.4 Bioactive compound and antioxidant activities

The total phenolic content in legume seeds is one of the important parameters indicating the potential antioxidant activities of seeds (Amarowicz et al., 2004). Findings from this study indicated that the radical scavenging activities for cooked micronised soft porridge of whole samples increased and in cooked micronised soft porridge of dehulled samples reduced (Table

4.8). It is important to point out that the increase in radical scavenging activities of the extracts may be due to the phenolic compounds becoming more extractable and the decrease may presumably be due to the phenolic compounds becoming less extractable. Micronisation causes physicochemical and microstructural changes in bambara groundnut seeds, the increased disintegration of middle lamella as well as separation of bambara groundnut parenchyma cell indicates that the integrity of the cell wall structure was interrupted, and possibly resulting in release of phenolics linked to the cell wall component. Therefore, this may result in the increase in total phenolic compound observed in cooked micronised whole soft porridge as also reported by Kayitesi (2013) on some phenolic compound such as phenolic acids in cooked micronised whole cowpea.

In extracts of dehulledbambara groundnut cooked soft porridge samples, the total phenolic compound and antioxidant properties decreased with micronisation (Table 4.8). The reduction in total phenolic content and antioxidant activities may be due to polymerisation, oxidation of phenolic compounds and thermal degradation of the phenolic compounds, (Taylor & Duodu, 2015) and phenolic-protein interactions (Rawelet al., 2005; Kayitesi, 2013). These changed the phenolic compounds into other forms such as oxidative products and phenolic-protein complexes which may have either low or no antioxidant activities (Arts et al., 2001).

The difference in the total phenolic contents and antioxidant activities results between the whole and dehulledbambara groundnut sample was probably be because of the absence of the seed coats in dehulled samples. Phenolic compounds are reported to mainly concentrate in the seed coat (Aparicio-Fernandez et al., 2005). Another reason may be due to micronisation increasing formation of complexes in the dehulled micronised bambara groundnut samples. The reduction in total phenolic compound and antioxidant properties in micronised (cooked) cowpea samples was attributed to the interaction of cowpea phenolic compounds with macromolecules such as proteins which may lead to the formation of insoluble complexes (Kayitesi, 2013). Therefore formation of phenolic-protein complexes may then have caused a reduction in extractability of phenolic compound in extracts of cooked soft porridge from dehulled micronised samples.

The decrease in the NSI (increase in thermally denatured protein) with micronisation in this study also related well with possible interaction with the phenolic compound in cooked bambara groundnut porridge. Protein form complexes with phenolic compounds through hydrogen bonding and hydrophobic interactions (Labuckas et al., 2008; Duodu et al., 2003). Thermal

processing may cause complex chemical reaction between phenolics and protein forming Maillard reaction products (Davies et al. 1998) and phenolic-protein interaction could either increase or restrict extraction of phenolic compound (Hachibamba et al., 2013). Phenolic compound are found either in free or bound to protein (Bravo et al., 1994) and free form phenolics are located in pericarps, testa and aleurone layer of kernel (Dykes & Rooney, 2006). The increased in the total phenolic compound and antioxidant properties (whole samples) in this study suggested possible release of free phenolics located in the seedcoat with micronisation. However, with absence of seed coat (dehulled samples) the decreased NSI suggested possible interaction between thermally denatured protein and phenolic compounds (bound). Therefore, this restricted the release of the phenolic compound during the extraction of the cooked micronised dehulled porridge samples.

5.3 Future research work

Complementary food for infant are majorly produced from cereal based plants such as maize, millet and sorghum (Temba et al., 2016).When the flour is heated in water, the starch swells and becomes highly viscous making it not suitable for their consumption (Kikafunda et al., 2006; Kikafunda et al., 1997). To avoid this problem in common practice, porridge is diluted with water which further may reduce the nutritient content and energy density of the foods (Kikafunda et al., 2006; Rombo et al., 2001). Table 5.3 presents comparison of rheological and nutritional properties of different cereal/tuber-legume complementary porridge with the cooked soft micronised bambara groundnut porridge.

Based on the low viscosity (0.05-0.18) of the cooked micronised soft porridge compared to the cereal/tuber-legume complementary porridge, the resulting flour of pre-soaked micronised bambara groundnut can make a useful blend with other food systems such as cereal and tubers.Low viscosity in complementary foods allows incorporation of more solids in mixture leading to an increase in nutrient density of the gruel (Bazaz et al, 2016). Therefore, incorporation of more solids (10% to 25%) of the resulting flour from micronised bambara groundnut would increase the protein energy density.

Table 5.3 Comparison of rheological and nutritional properties of different cereal/tuber-legumes complementary porridge

Types of Complementary porridge	[±]Viscosity (mPas)	[*]EGI	[×]IVPD (%)
[1]Extruded Cassava-Soy	2.3	104	93
[2]Roasted Pearl millet-cowpea	2.7	nd	43
[2]Roasted Teff- Pearl millet-cowpea	2.0	nd	44
[3]Pre-soaked micronised bambara groundnut	0.05-0.18	75-80	89-90
[3]Pre-soaked unmicronisedbambara groundnut	0.18	70- 72	88-89

[±]Viscosity: [2]Griffth et al., 1998 (1 s^{-1} shear rate); [3]In the current study and [1]Muoki et al., 2012 (100s^{-1} shear rate).

Flour blend percentage: 65% cassava flour and 35% defatted soy flour, 60% Pearl millet and 40% cowpea, 20%Teff, 40% and 40% Pearl millet-cowpea, 100% flour from pre-soaked micronised bambara groundnut.

Porridge total solid: Extruded cassava-defatted soy flour (25%), Roasted Pearl millet-cowpea; Roasted Teff-Pearl millet-cowpea (15%), pre-soaked micronised bambara groundnut.

[*]EGI= Estimated glycemic index.

[×]IVPD= in vitro protein digestibility.

Micronisation of pre-soaked bambara groundnut and wet heat cooking increased the nutritional properties in terms of digestibility of starch and protein in their cooked soft porridge. Protein digestibility-corrected amino acid score (PDCAAS) and gross energy density measures the protein and energy quality of complementary porridge (Muoki et al 2012; Ejigui et al., 2007; Kikafunda et al., 2006). This determines the nutritional quality of different starchy-protein based complementary infant foods and if the complementary porridge meets the recommended protein energy standard for the infant feeding (Muoki et al 2012). Thus for future work, one can investigate PDCAAS and gross energy density of the resulting flour of pre-soaked micronised bambara groundnut or its blend with other starchy based flour.

This study also indicated the extracts from cooked micronisedbambara groundnut porridge exhibited radical scavenging activities. So it can be said to have some potential health promoting properties. Similarly, cooked porridges from bean flour (Goderska et al., 2008), common beans (Akillioglu&Karakaya, 2010) and cowpea (Nderitu et al., 2013; Hachibamba et al., 2013)have also been reported possessed high total phenolic content and exhibited radical scavenging activities compared to the control samples.In order to determine the bioaccessible fractions of the phenolic compounds in the porridge in the body system, *in vitro* or *in vivo* digestion of the samples can be conducted to test its inhibitory effect on DNA damage and copper catalysed LDL oxidation (Nderitu et al. 2013; Hachibamba et al., 2013).

6 CONCLUSIONS AND RECOMMENDATIONS

The reduction in cooking time of pre-soaked micronised bambara groundnut seeds (53%, moisture) is attributed to development of solubilisation of pectin, starch distruption and protein denaturation. Micronisation (130 °C, 5, 10 and 15 min) produces fissures in seed coats and cotyledons, which leads to improvement in water uptake and rate during cooking. The production of micronised bambara groundnut with reduced cooking time provide an opportunity for utilisation of bambara groundnut as a shop shelves dry grains product especially for urban consumers whose busy life style makes convenience an important factor in their food choices.Dehulling of bambara groundnut prior to infrared heating result in splitting of the seeds. Therefore, it is recommended that dehulled bambara groundnut seeds should not be used for micronisation processing if whole bambara groundnut are desired after cooking. However, dehulled bambara groundnut susceptible to splitting can be micronised for processing into products such as flour.

The changes in physicochemical properties ofbambara groundnut starch and proteins affect their functionality, resulting in increasing flour swelling and reducing water solubility power. The mechanism of reduction in pasting viscosity of flours from micronised pre-soaked (53% moisture, 130 °C) bambara groundnuts has been explained in this thesis. This mechanism involves the interplay of aggregates of starch granules and the protein matrix. The starch granules in pre-soaked and micronised bambara groundnut seeds undergo pre-gelatinisation process under the mild micronisation conditions (130 °C) as evidenced by reduction in enthalpy energy required. Simultaneously, micronisation of pre-soaked bambara groundnuts denatures protein and induces the unfolding of protein by exposing hydrophobic sites thus increasing the surface hydrophobicity of the proteins as shown by reduction in nitrogen solubility index (NSI). The possible increased aggregations of partially gelatinised starch embedded in denatured protein matrices in pre-soaked micronised bambara groundnut (130 °C, 5, 10 and 15 min), contributes to prevention starch molecules dispersion and entanglement, thus reduction in viscosity.

The flours from pre-soaked bambara groundnuts micronised to mild temperatures (130 °C) with reduced viscosity and were shear thinning as measured in this study will have its application in food systems where low viscosity and high energy protein dense ingredients is desired. It is

recommended that flours should be incorporated in a complementary food system such as cereal and tuber in order to elucidate the impact and extent of the modification of these components on the functionality of the flour- based product.

The cooked porridges of micronised whole bambara groundnut samples in this study increased in their total extractable phenolics and radical scavenging properties. Although micronisation decreases total extractable phenolics of cooked porridges of dehulled bambara groundnut samples due to the absence of seed coats and interaction between phenolic and other bioactive compounds with the legume protein, the porridges still posses radical scavenging properties with health promoting benefit.

This study has shown that micronisation of either whole or dehulled pre-soaked bambara groundnut seeds to mild temperature through its effects on the physical structure and modification of starch, protein and pectin can be used as a pre-treatment of bambara groundnut seeds to produce bambara groundnut seeds with shorter cooking time and instant resulting flour with modified functionality, nutritional and potential bioactive properties. As such micronisation of pre-soaked seeds could be one of the alternative processes for improving the utilisation for bambara groundnut seeds.

7 REFERENCES

Abdul-Kadir, R., Bargman, T. J., & Rupnow, J. H., 2006. Effect of infrared heat processing on rehydration rate and cooking of *Phaseolus vulgaris (var. Pinto).Journal of Food Science*,55, 1472-1473.

Abu, J. O., & Minnaar, A., 2009. Gamma irradiation of cowpea *(Vigna unguiculata L. Walp)* seeds: effect on colour, cooking quality and pasting characteristics. *International Journal of Food Science & Technology*, 44, 2335-2341.

Adebowale, K., & Lawal, O., 2002. Effect of annealing and heat moisture conditioning on the physicochemical characteristics of bambara groundnut *(Voandzeia subterranea)* starch. *Food/Nahrung*, 46, 311-316.

Adebowale, K. O., Afolabi, T. A., & Lawal, O. S., 2002. Isolation, chemical modification and physicochemical characterisation of bambara groundnut *(Voandzeia subterranean)* starch and flour. *Food Chemistry*, 78, 305-311.

Adebowale, Y. A., Schwarzenbolz, U., & Henle, T., 2011. Protein isolates from bambara groundnut *(Voandzeia Subterranean L.)*: Chemical characterization and functional properties. *International Journal of Food Properties*, 14, 758-775.

Aguilera, J., & Stanley, D., 1985. A review of textural defects in cooked reconstituted legumes–the influence of storage and processing. *Journal of Food Processing and Preservation*, 9, 145-169.

Ainsworth, E. A., & Gillespie, K. M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using *folin–ciocalteu* reagent.*Nature protocols*, 2, 875-877.

Akillioglu, H. G., & Karakaya, S., 2010. Changes in total phenols, total flavonoids, and antioxidant activities of common beans and pinto beans after soaking, cooking, and *in vitro* digestion process.*Food Science and Biotechnology*, 19, 633-639.

Alajaji, S. A., & El-Adawy, T. A., 2006. Nutritional composition of chickpea *(Cicer arietinum L.)* as affected by microwave cooking and other traditional cooking methods.*Journal of Food Composition and Analysis*, 19, 806-812.

Alobo, A. P., 1999. Production and organoleptic assessment of akara from bambara groundnut *(Voandzeia subterranea (L.) Thouars)*. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*, 53, 313-320.

Alonso, R., Aguirre, A., & Marzo, F., 2000. Effects of extrusion and traditional processing methods on antinutrients and *in vitro* digestibility of protein and starch in faba and kidney beans. *Food Chemistry*, 68, 159-165.

Alozie, Y. E., Iyam, M. A., Lawal, O., Udofia, U., & Ani, I. F., 2009. Utilisation of bambara groundnut flour blends in bread production. *Journal of Food Technology*, 7, 111-114.

Amarowicz, R., Pegg, R., Rahimi-Moghaddam, P., Barl, B., & Weil, J., 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, 84, 551-562.

Amarteifio, J. O., & Moholo, D., 1998. The Chemical composition of four legumes consumed in botswana. *Journal of Food Composition and Analysis*, 11, 329-332.

Anchirinah, V.M., Yiridoe, E.K., & Bennett-Lartey S.O., 2001. Enhancing sustainable production and genetic resource conservation of Bambara groundnut: A survey of indigenous agricultural knowledge systems.*Outlook on Agriculture*, 30:281-8.

Andrejko, D., Rydzak, L., Slaska-Grzywna, B., Goÿdziewska, M., & Kobus, Z., 2008. Influence of preliminary thermal processing applying infra-red radiation on pea seeds cooking process. *International Agrophysics*, 22, 17-20.

Annan, N., Plahar, W., & Swetman, T., 2002. Effect of kawe treatment on the water absorption and cooking characteristics of bambara groundnut varieties. *Food Research Institute (FRI)*, Accra, Ghana. Pg 15.

Aparicio-Fernandez, X., Yousef, G. G., Loarca-Pina, G., De Mejia, E., & Lila, M. A., 2005. Characterization of polyphenolics in the seed coat of black jamapa bean *(Phaseolus vulgaris L.)*. *Journal of Agricultural and Food Chemistry*, 53, 4615-4622.

Apata, D., & Ologhobo, A., 1994. Biochemical evaluation of some Nigerian legume seeds. *Food Chemistry*, 49, 333-338.

Aremu, M. O., Olaofe, O., & Akintayo, T. E., 2006.A comparative study on the chemical and amino acid composition of some Nigerian under-utilized legume flours.*Pakistan Journal of Nutrition*, 5, 34-38.

Aremu, M., Olaofe, O., & Akintayo, E., 2007. Functional properties of some Nigerian varieties of legume seed flours and flour concentration effect on foaming and gelation properties. *Journal of Food Technology*, 5, 109-113.

Arnao, M. B., Cano, A., & Acosta, M., 2001. The hydrophilic and lipophilic contribution total antioxidant activity. *Food Chemistry*,73, 239-244.

Arntfield, S. D., Scanlon, M. G., Malcolmson, L. J., Watts, B. M., Cenkowski, S., Ryland, D., & Savoie, V., 2001. Reduction in lentil cooking time using micronisation: Comparison of two micronization temperatures. *Journal of Food Science*,66, 500-505.

Arntfield, S. D., Scanlon, M. G., Malcolmson, L. J., Watts, B., Ryland, D., & Savoie, V., 1997. Effect of tempering and end moisture content on the quality of micronized lentils. *Food Research International*, 30, 371-380.

Arts, M. J., T. J., Haenen, G. R.M.M., Voss, H.P., & Bast, A., 2001. Masking of antioxidant capacity by the interaction of flavonoids with protein. *Food and Chemical Toxicology*, 39,787-791.

Autio, K., & Salmenkallio-Marttila, M., 2001. Light microscopic investigations of cereal grains, doughs and breads. *LWT-Food Science and Technology*, 34, 18-22.

Awika, J. M., & Rooney, L. W., 2004.Sorghum phytochemicals and their potential impact on human health.*Phytochemistry*, 65, 1199-1221.

Awika, J.M., Dykes, L., Gu, L., Rooney, L.W. & Prior, R.L., 2003. Processing of sorghum *(Sorghum bicolor)* and sorghum products alters procyanidin oligomer and polymer distribution and content. *Journal of Agricultural and Food Chemistry*, 51, 5516–5521.

Azam-Ali, S., Sesay, A., Karikari, S., Massawe, F., Aguilar-Manjarrez, J., Bannayan, M. & Hampson, K. 2001. Assessing the potential of an underutilized crop–a case study using bambara groundnut. *Experimental Agriculture*, 37, 433-472.

Bandyopadhyay, S., Peralta-Videa, J. R. & Gardea-Torresdey, J. L. 2013. Advanced analytical techniques for the measurement of nanomaterials in food and agricultural samples: a review. *Environmental Engineering Science*, 30, 118-125.

Barimalaa, I. S., Agoha, G., Oboh, C. A., & Kiin-Kabari, D. B., 2005. Studies on bambara groundnut flour performance in okpa preparation. *Journal of the Science of Food and Agriculture*, 85, 413-417.

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

Baudoin, J. P., & Mergeai, G., 2001. Grain legumes in crop production in tropical Africa. Pg 313 – 317.

Bazaz, R., Baba, W.N., & Masoodi, F.A., 2016. Development and quality evaluation of hypoallergic complementary foods from rice incorporated with sprouted green gram flour. *Cogent Food & Agriculture*, 2, http://dx.doi.org/10.1080/23311932.2016.1154714

Bean, S., Chung, O., Tuinstra, M., Pedersen, J., & Erpelding, J., 2006. Evaluation of the single kernel characterization system (SKCS) for measurement of sorghum grain attributes. *Cereal chemistry*, 83, 108-113.

Bellido, G., Arntfield, S. D., Cenkowski, S., & Scanlon, M., 2006. Effects of micronization pretreatments on the physicochemical properties of navy and black beans *(Phaseolus vulgaris L.). LWT - Food Science and Technology*, 39, 779-787.

Bernal-Lugo, I., Parra, C., Portilla, M., Peña-Valdivia, C. B. & Moreno, E., 1997. Cotyledon thermal behavior and pectic solubility as related to cooking quality in common beans. *Plant Foods for Human Nutrition*, 50, 141-150.

Boisen, S., & Eggum, B., 1991. Critical evaluation of *in vitro* methods for estimating digestibility in simple-stomach animals. *Nutrition Research Reviews*, 4, 141-162.

Bond, J. S., & Beynon, R. J., 1987. Proteolysis and physiological regulation. *Molecular Aspects of Medicine*, 9, 173-287.

Borget, M., 1992. Food legumes. in: The tropical agriculturalist, CTA Macmillan. 2.

Bornhorst, G. M., & Singh, R.P., 2012. Bolus formation and disintegration during digestion of food carbohydrates. *Comprehensive Reviews in Food Science and Food Safety*, 11:101-118.

Brand-Miller, J.C., Holt, S.H.A., Pawlak, D.B., & Joanna, M., 2002. Glycemic index and obesity. *American Journal of Clinical Nutrition*, 76: 281S-285S.

Bravo, L., Abia, R., & Saura-Calixto, F., 1994. Polyphenols as dietary fiber associated compounds. Comparative study on *in vivo* and *in vitro* properties.*Journal of Agricultural and Food Chemistry*, 42, 1481-1487.

Brooke, B.E. 1991. The study of food systems using confocal laser scanning light microscopy.*Microscopy and Analysis*, 26, 13-15.

Brough, S., & Azam‐Ali, S., 1992. The effect of soil moisture on the proximate composition of Bambara groundnut *(Vigna subterranea (L) Verdc).Journal of the Science of Food and Agriculture*, 60, 197-203.

Brough, S., Azam-Ali, S., & Taylor, A., 1993. The potential of bambara groundnut *(Vigna subterranea)* in vegetable milk production and basic protein functionality systems. *Food Chemistry*, 47, 277-283.

Carman, K., 1996. Some physical properties of lentil seeds.*Journal of Agricultural Engineering Research*, 63, 87-92.

Cenkowski, S., & Sosulski, F., 1998. Cooking characteristics of split peas treated with infrared heat. *Transactions of the ASAE*, 41, 715-720.

Cenkowski, S., & Sosulski, F. W., 1997. Physical and cooking properties of micronized lentils.*Journal of Food Process Engineering*, 20, 249-264.

Cenkowski, S., Arntfield, S., & Scanlon, M., 2007. Far Infrared dehydration and processing.*Food Drying Science and Technology: Microbiology, Chemistry, Application*, 157.

Cenkowski, S., Hong, J.T., Scanlon, M., & Arntfield, S., 2003. Development of a mathematical model for high-intensity infrared processing (micronisation) of peas. *Transactions of the ASAE*, 46, 705-713.

Chen, C.R., & Ramaswamy, H.S., 1999. Rheology of tapioca starch. *Food Research International*, 32, 319–325.

Chibarabada, T. P., Modi, A. T., & Mabhaudhi, T., 2014. Seed quality characteristics of a bambara groundnut *(Vigna subterranea L.)* landrace differing in seed coat colour. *South African Journal of Plant and Soil*, 31, 219-226.

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., & Larondelle, Y., 2007. Optimization of extraction conditions of antioxidant phenolic compounds from mashua *(Tropaeolum tuberosum Ruíz and Pavón)* tubers. *Separation and Purification Technology*, 55, 217-225.

Coultate, T.P., 2002. *Food: The chemistry of its components*. Cambridge: The royal cowpeas. Journal of *Agriculture and Food Chemistry*, 40, 2483-2487.

Crosbie, G. B., & Ross, A. S., 2007. *The RVA handbook, American Association of Cereal Chemists*, Inc (AACC).

Damodaran, S., 1996. Functional properties.In S. Nakai and H.W. Modler. *Food Protein: Properties and Characterisation*. New York: Wiley-VCH. Pp 167-234.

Davies, C., Netto, F., Glassenap, N., Gallaher, C., Labuza, T., & Gallaher, D., 1998. Indication of the maillard reaction during storage of protein isolates. *Journal of Agricultural and Food Chemistry*, 46, 2485-2489.

Deepa, C., & Hebbar, H. U., 2016. Effect of high-temperature short-time 'Micronization'of grains on product quality and cooking characteristics. *Food Engineering Reviews*, 1-13.

Demooy, B., & Demooy, C., 1990. Evaluation of cooking time and quality of seven diverse cowpea *(Vigna unguiculata (L.)Walp.)* varieties. *International Journal of Food Science & Technology*, 25, 209-212.

Duodu, K., Taylor, J., Belton, P., & Hamaker, B., 2003. Factors affecting sorghum protein digestibility. *Journal of Cereal Science*, 38, 117-131.

Dürrenberger, M. B., Handschin, S., Conde-Petit, B., & Escher, F., 2001. Visualization of food structure by confocal laser scanning microscopy (CLSM).*LWT-Food Science and Technology*, 34, 11-17.

Dykes, L., & Rooney, L. W., 2006. Sorghum and millet phenols and antioxidants.*Journal of Cereal Science*, 44(3), 236–251.

Dykes, L., Rooney, L. W., Waniska, R. D., & Rooney, W. L., 2005. Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. *Journal of Agricultural and Food Chemistry*, 53, 6813-6818.

Ejigui, J., Savoie, L., Marin, J., & Desrosiers, T., 2007. Improvement of the nutritional quality of a traditional complementary porridge made of fermented yellow maize *(Zea mays)*:

Effect of maize–legume combinations and traditional processing methods. *Food and nutrition bulletin*, 28, 23-34.

Eltayeb, A. R. S., Ali, A. O., Abou-Arab, A. A., & Abu-Salem, F. M., 2011. Chemical composition and functional properties of flour and protein isolate extracted from bambara groundnut *(Vigna subterranean)*. *African Journal of Food Science*, 5, 82-90.

Englyst, H. N., Kingman, S. M., Hudson, G. J., & Cummings, J. H., 1996. Measurement of resistant starch *in vitro* and *in vivo*. *British Journal of Nutrition*, 75,749-755.

Englyst, K.N., Vinoy, S., Englyst, H.N., & Langa, V., 2003. Glycaemic Index of cereal products explained by their content of rapidly and slowly available glucose. *British Journal of Nutrition*, 89: 329-339.

Enwere, N., & Hung, Y.C., 1996. Some chemical and physical properties of bambara groundnut *(Voandzeia subterranea Thouars)* seed and products. *International Journal of Food Sciences and Nutrition*, 47, 469-475.

Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B., 2010. Thorough study of reactivity of various compound classes toward the *folin–ciocalteu* reagent. *Journal of Agricultural and Food Chemistry*, 58, 8139-8144.

Fasina, O., Tyler, B., Pickard, M., Zheng, G.H., & Wang, N., 2001. Effect of infrared heating on the properties of legume seeds. *International Journal of Food Science & Technology*, 36, 79-90.

Fasina, O., Tyler, R., Pickard, M., & Zheng, G., 1999. Infrared heating of hulless and pearled barley. *Journal of Food Processing and Preservation*, 23, 135-151.

Fasoyiro, S., Ajibade, S., Omole, A., Adeniyan, O., & Farinde, E., 2006. Proximate, minerals and antinutritional factors of some underutilized grain legumes in south-western Nigeria. *Nutrition & Food Science*, 36, 18-23.

Fery, R. L., 2002. New opportunities in *Vigna*. *Trends in new crops and new uses***,** 424-428.

Food & Agriculture Organization of United Nations, 2004. Value of production: Bambara groundnut (accessed online at). http://faostat3.fao.org/crop/bambara_groundnut.htm (September 2016).

Giami, S. Y., & Okwechime, U. I., 1993. Physicochemical properties and cooking quality of four new cultivars of Nigerian cowpea *(Vigna unguiculata L walp)*. *Journal of the Science of Food and Agriculture*, 63, 281-286.

Ginzburg, A. S. 1969. *Application of Infra–Red Radiation in Food Processing*. London, U.K.: Leonard Hill.

Goderska, K., Gumienna, M., & Czarnecki, Z., 2008.Release of phenolic compounds from bean flour, bean-derived chips and black chokeberry juice and changes in their antioxidant activity during digestion in an *in vitro* gastrointestinal model.*Polish Journal of Food and Nutrition Sciences*, 58, 497-501.

Gonçalves, E., Pinheiro, J., Abreu, M., Brandão, T., & Silva, C. L., 2007. Modelling the kinetics of peroxidase inactivation, colour and texture changes of pumpkin *(Cucurbita maxima L.)* during blanching. *Journal of Food Engineering*, 81, 693-701.

Goni, I., Garcia-Alonso, A., & Saura-Calixto, F., 1997.A starch hydrolysis procedure to estimate glycemic index.*Nutrition Research*,17, 427–437.

Goode, D. L., Wiltschko, E. A., Ulmer, H. M. & Arendt, E. K., 2005. Application of the rapid visco analyser as a rheological tool for the characterisation of mash viscosity as affected by the level of barley adjunct. *Journal of the Institute of Brewing*, 111, 165-175.

Grant, E. & Halstead, B. J. 1998. Dielectric parameters relevant to microwave dielectric heating.*Chemical Society Reviews*, 27, 213-224.

Greve, L. C., Shackel, K. A., Ahmadi, H., McArdle, R. N., Gohlke, J. R., & Labavitch, J. M., 1994. Impact of heating on carrot firmness: contribution of cellular turgor. *Journal Agriculture and Food Chemistry*, 42, 2896–2899.

Griffith, L.D., Castell-Perez, M.E., & Griffith, M.E., 1998. Effect of blend and processing method on the nutritional quality of weaning foods made from select cereals and legumes. *Cereal Chemistry*, 75 (1), 105–12.

Hachibamba, T., Dykes, L., Awika, J., Minnaar, A., & Duodu, K. G., 2013. Effect of simulated gastrointestinal digestion on phenolic composition and antioxidant capacity of cooked cowpea *(Vigna unguiculata)* varieties. *International Journal of Food Science & Technology*, 48, 2638-2649.

Hamaker, B. R., Kirleis, A. W., Mertz, E.T., & Axtell, J. D., 1986. Effect of cooking on protein profiles and *in vitro* digestibility of sorghum and maize. *Journal of Agricultural and Food Chemistry*, 34, 647-659.

Hasjim, J., Lavau, G.L., Gidley, M.J., & Gilbert, R.G., 2010. *In vivo* and *in vitro*starch digestion: Are current *in vitro* technique adequate? *Biomolecules*, 11: 3600-3608.

Hood, L. 1982. Current concepts of starch structure. *Food Carbohydrates*, 218-224.

Heertje, I., Vlist, P., Blonk, J., Hendrickx, H.,& Brakenhoff, G., 1987. Confocal scanning laser microscopy in food research: some observations. *Food Structure*, 6, 2.

Hsu, H., Vavak, D., Satterlee, L. & Miller, G., 1977.A multienzyme technique for estimating protein digestibility.*Journal of Food Science*, 42, 1269-1273.

Huma, N., Anjum, M., Sehar, S., Khan, M. I., & Hussain, S., 2008. Effect of soaking and cooking on nutritional quality and safety of legumes.*Nutrition & Food Science*, 38, 570-577.

Hur, S. J., Lim, B. O., Decker, E. A., & Mcclements, D. J., 2011. *In vitro* human digestion models for food applications. *Food Chemistry*, 125, 1-12.

Isik, E. 2007a. Some physical and mechanical properties of round red lentil grains.*Applied Engineering in Agriculture*, 23, 503-509.

Isik, E. 2007b. Moisture dependent physical and mechanical properties of green laird lentil *(Lens culinaris)* grains. *Pakistan Journal of Biological Sciences*, 10, 474-480.

Jackson, G.M., & Varriano-Marston, E., 1981. Hard-to-cook phenomenon inbeans: Effects of accelerated storage on water absorption and cooking time. *Journal of Food Science*, 46, 799-803.

Jenkins, D.J.A., Thomas, M.S.W., & Taylor, R.H., 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition*, 34: 362-366.

Jideani, V. A., & Diedericks, C. F., 2014.Nutritional, therapeutic, and prophylactic properties of *Vigna subterranea* and *Moringa oleifera*.*Antioxidant-Antidiabetic Agents and Human Health*, 187.

Kabas, O., Yilmaz, E., Ozmerzi, A., & Akinci, İ., 2007. Some physical and nutritional properties of cowpea seed *(Vigna sinensis L.)*. *Journal of Food Engineering*, 79, 1405-1409.

Kaptso, K. G., Njintang, Y. N., Nguemtchouin, M. M. G., Scher, J., Hounhouigan, J. & Mbofung, C. M. 2015. Physicochemical and micro-structural properties of flours, starch and proteins from two varieties of legumes: bambara groundnut *(Vigna subterranea)*. *Journal of Food Science and Technology*, 52, 4915-4924.

Kaptso, K., Njintang, Y., Komnek, A., Hounhouigan, J., Scher, J. & Mbofung, C. 2008. Physical properties and rehydration kinetics of two varieties of cowpea *(Vigna unguiculata)*and bambara groundnuts *(Voandzeia subterranea)* seeds. *Journal of Food Engineering*, 86, 91-99.

Kayitesi, E. 2013. Micronisation of cowpeas: Effects on sensory quality, phenolic compounds and bioactive properties.University of Pretoria, Pretoria.

Kayitesi, E., Duodu, K. G., Minnaar, A., & De Kock, H. L., 2012. Effect of micronisation of pre-conditioned cowpeas on cooking time and sensory properties of cooked cowpeas.*Journal of the Science of Food and Agriculture*, 93, 838-845.

Khattab, R., & Arntfield, S., 2009. Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors.*LWT-Food Science and Technology*, 42, 1113-1118.

Khattab, R. Y., Arntfield, S. D., & Nyachoti, C. M., 2009. Nutritional quality of legume seeds as affected by some physical treatments, Part 1: Protein quality evaluation. *LWT-Food Science and Technology*, 42, 1107-1112.

Kiin-Kabari, D. B., & Giami, S. Y., 2015. Physico chemical properties and *in-vitro* protein digestibility of Non-wheat cookies prepared from plantain flour and bambara groundnut protein concentrate. *Journal of Food Research*, 4, 78.

Kikafunda, J. K., Abenakyo, L., & Lukwago, F. B., 2006. Nutritional and sensory properties of high energy/nutrient dense composite flour porridges from germinated maize and roasted beans for child-weaning in developing countries: a case for Uganda. *Ecology of Food and Nutrition*, 45, 279-294.

Kikafunda, J. K., Walker, A. F., & Abeyasekera, S., 1997. Optimising viscosity and energy density of maize porridges for child weaning in developing countries.*International Journal of Food Sciences and Nutrition*, 48, 401-409.

Kiosseoglou, V. & Paraskevopoulou, A., 2011. Functional and physicochemical properties of pulse proteins. In Tiwari, B. K., Gowen, A. & Mckenna, B. (Eds). *Pulse Foods: Processing, Quality and Nutraceutical Applications, vol 1(pp 57) Elsevier Inc., London.*Academic Press.

Krishnamurthy, K., Khurana, H. K., Soojin, J., Irudayaraj, J., & Demirci, A., 2008. Infrared Heating in Food Processing: An Overview. *Comprehensive Reviews in Food Science and Food Safety*, 7, 2-13.

Kudre, T. G., Benjakul, S., & Kishimura, H., 2013. Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *Journal of the Science of Food and Agriculture*, 93, 2429-2436.

Labuckas, D. O., Maestri, D. M., Perelló, M., Martínez, M. L., & Lamarque, A. L., (2008).Phenolics from walnut *(Juglans regia L.)* kernels: Antioxidant activity and interactions with proteins. *Food Chemistry*, 107, 607–612.

Lacroix, B., Assoumou, Y., & Sangwan, R., 2003. Efficient *in vitro* direct shoot organogenesis and regeneration of fertile plants from embryo explants of Bambara groundnut *(Vigna subterranea L. Verdc.).Plant Cell Reports*, 21, 1153-1158.

Liu, K., Hung, Y.C., & Phillips, R.D., 1993. Mechanism of Hard-to-Cook defect in cowpeas: Verification via microstructure examination. *Food structure*,12, 51-58.

MacDonald-Wicks, L. K., Wood, L. G. & Garg, M. L. 2006. Methodology for the determination ofbiological antioxidant capacity *in vitro*: a review. *Journal of the Science of Food andAgriculture*, 86, 2046-2056.

Mckenna, A. B. 1997. Examination of whole milk powder by confocal laser scanning microscopy.*Journal of Dairy Research*, 64, 423-432.

Mezger, T.G. (2006). The rheology handbook: for users of rotational and oscillatory rheometers, Pages 114-125. Germany: Hannover.

Michael, P., Ámingos, D., & Baghurst R., 1991. Tilden Lecture. Applications of microwave dielectric heating effects to synthetic problems in chemistry.*Chemical Society Reviews*, 20, 1-47.

Mpotokwane, S., Gaditlhatlhelwe, E., Sebaka, A., & Jideani, V., 2008. Physical properties of bambara groundnuts from Botswana. *Journal of Food Engineering*, 89, 93-98.

Mualikrishna, G., & Tharanathan, R. N., 1994. Characterization of pectic polysaccharides from pulse husks. *Food chemistry*, 50, 87-89.

Mubarak, A. 2005. Nutritional composition and antinutritional factors of mung bean seeds *(Phaseolus aureus)* as affected by some home traditional processes. *Food Chemistry*, 89, 489-495.

Mune, M., Minka, S. R., Mbome, I. L., & Etoa, F., 2011. Nutritional potential of Bambara bean protein concentrate. *Pakistan Journal of Nutrition*, 10, 112-119.

Muoki, P. N., 2013. Nutritional, rheological and sensory properties of extruded cassava-soy complementary porridges.University of Pretoria, Pretoria.

Muoki, P. N., De Kock, H. L., & Emmambux, M. N., 2012. Effect of soy flour addition and heat-processing method on nutritional quality and consumer acceptability of cassava complementary porridges.*Journal of the Science of Food andAgriculture*, 92, 1771-1779.

Muoki, P. N., Kinnear, M., Emmambux, M. N., & De Kock, H. L., 2015. Effect of the addition of soy flour on sensory quality of extrusion and conventionally cooked cassava complementary porridges. *Journal of the Science of Food and Agriculture*, 95, 730-738.

Mwangwela, A. M., 2006. Physicochemical characteristics of conditioned and micronised cowpeas and functional properties of the resultant flours.University of Pretoria, Pretoria.

Mwangwela, A. M., Waniska, R. D., & Minnaar, A., 2006. Hydrothermal treatments of two cowpea *(Vigna unguiculata L. Walp)* varieties: effect of micronisation on physicochemical and structural characteristics. *Journal of the Science of Food and Agriculture*, 86, 35-45.

Mwangwela, A. M., Waniska, R. D., & Minnaar, A., 2007b. Effect of micronisation temperature (130 and 170 ºC) on functional properties of cowpea flour.*Food chemistry*, 104, 650-657.

Mwangwela, A. M., Waniska, R. D., Mcdonough, C. & Minnaar, A. 2007a. Cowpea cooking characteristics as affected by micronisation temperature: a study of the physicochemical and functional properties of starch. *Journal of the Science of Food and Agriculture*, 87, 399-410.

Nderitu, A. M., Dykes, L., Awika, J. M., Minnaar, A., & Duodu, K. G., 2013. Phenolic composition and inhibitory effect against oxidative DNA damage of cooked cowpeas as affected by simulated *in vitro* gastrointestinal digestion. *Food Chemistry*, 141(3), 1763–1771.

Ndungu, K. E., Emmambux, M. N., & Minnaar, A., 2012. Micronisation and hot air roasting of cowpeas as pretreatments to control the development of hard-to-cook phenomenon. *Journal of the Science of Food and Agriculture*, 92, 1194-1200.

Newkirk, R., 2010. Soybean: Feed industry guide. *Canadian International Grains Institute.*

Nnam, N., 2001a. Chemical, sensory and rheological properties of porridges from processed sorghum *(Sorghum bicolor, bambara groundnut (Vigna subterranea L. Verdc)* and sweet potato *(Ipomoea batatas)* flours. *Plant foods for human nutrition*, 56, 251-264.

Nnam, N., 2001b. Comparison of the protein nutritional value of food blends based on sorghum, bambara groundnut and sweet potatoes. *International Journal of Food Sciences and Nutrition*, 52, 25-29.

Nti, C. A., 2009. Effects of bambara groundnut *(Vigna subterranea)* variety and processing on the quality and consumer appeal for its products. *International Journal of Food Science & Technology*, 44, 2234-2242.

Nyau, V., Prakash, S., Rodrigues, J., & Farrant, J., 2015. Antioxidant activities of Bambara groundnuts as assessed by FRAP and DPPH assays. *American Journal of Food and Nutrition*, 3, 7-11.

Ojimelukwe, P. C. 1999. Cooking characteristics of four cultivars of bambara groundnut seeds and starch isolate. *Journal of Food Biochemistry*, 23, 109-117.

Okonkwo, S. & Opra, M. 2010. The Analysis of Bambara Nut *(Voandzeia subterranean (L.) Thouars)* for Sustainability in Africa.*Research Journal of Applied Science*, 5, 394-396.

Onimawo, I., Momoh, A. & Usman, A. 1998. Proximate composition and functional properties of four cultivars of bambara groundnut *(Voandezeia subterranea)*. *Plant Foods for Human Nutrition*, 53, 153-158.

Opoku, A., Tabil, L., Sundaram, J., Crerar, B., & Park, S., 2003. Conditioning and dehulling of pigeon peas and mung beans. *CSAE/SCGR Paper*.

Oyeleke, G., Afolabi, O., & Isola, A., 2012. Some quality characteristics and carbohydrate fractions of bambara groundnut *(Vigna subterranea L.)* seed flour. *Journal of Applied Chemistry*, 4, 16-19.

Penicela, L., 2011. The Influence of seed coat and cotyledon structure on cooking characteristics of cowpea. MSc dissertation. (Food Science).University of Pretoria.

Peroni, F.G., Rocha, T.S., & Franco, C.M.L., 2006. Some structural and physicochemical characteristics of tuber and root starches. *Food Science &TechnologyInternational*, 12,505–513.

Phadi, M. M., 2004. Physico-chemical Effects of Micronization on Cowpeas *(Vigna Unguiculata L. Walp)*. *MInst Agrar Desssertation*, University of Pretoria.

Poulter, N., 1981. Properties of some protein fractions from Bambara *(Vigna subterrranean (L) Verdc)* groundnut.*Journal of the Science of Food andAgriculture*, 32, 44-50.

Prinyawiwatkul, W., Beuchat, L. R., Mcwatters, K. H., & Phillips, R. D., 1997. Functional properties of cowpea *(Vigna unguiculata)* flour as affected by soaking, boiling, and fungal fermentation. *Journal of Agricultural and Food Chemistry*, 45, 480-486.

Prior, R. L., Wu, X., & Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53, 4290-4302.

Proctor, J., & Watts, B., 1987. Development of a modified Mattson bean cooker procedure based on sensory panel cookability evaluation. *Canadian Institute of Food Science and Technology Journal*, 20, 9-14.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorizing assay. *Free Radical Biology and Medicine*, 26 (9/10), 1231-1237.

Rawel, H. M., Meidtner, K., & Kroll, J., 2005. Binding of selected phenolic compounds to proteins. *Journal of Agricultural and Food Chemistry*, 53, 4228-4235.

Rombo, G. O., Taylor, J., & Minnaar, A., 2001. Effect of irradiation, with and without cooking of maize and kidney bean flours, on porridge viscosity and *in vitro* starch digestibility.*Journal of the Science of Food and Agriculture*, 81, 497-502.

Rust, A., & Manga, M., 2002. Effects of bubble deformation on the viscosity of dilute suspensions. *Journal of Non-Newtonian Fluid Mechanics*, 104, 53-63.

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R., 2006. Resistant starch–a review. *Comprehensive Reviews in Food Science and Food Safety*, 5, 1-17.

Sakai, N., & Hanzawa, T., 1994. Applications and advances in far-infrared heating in Japan. *Trends in Food Science & Technology*, 5, 357-362.

Sandu, C., 1986. Infrared radiative drying in food engineering: a process analysis. *Biotechnology progress*, 2, 109-119.

Sarantinos, J., & Black, R., 1996. Effects of micronisation on the chemical and functional properties of chickpeas. *Food Australia: Official Journal of CAFTA and AIFST*.

Scanlon, M. G., Cenkowski, S., Segall, K. I., & Arntfield, S. D., 2005. The physical properties of micronised lentils as a function of tempering moisture.*Biosystems Engineering*, 92, 247-254.

Sefa-Dedeh, S., Stanley, D., & Voisey, P., 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpeas *(Vigna unguiculata). Journal of Food Science*, 43, 1832-1838.

Sharma, G., 2009. Micronisation. Defence Research and Development Organization, Ministry of Defence, Government of India. *Science Spectrum*, 169-171.

Shelembe, J. S., Cromarty, D., Bester, M., Minnaar, A., & Duodu, K. G., 2014. Effect of acidic condition on phenolic composition and antioxidant potential of aqueous extracts from sorghum *(Sorghum bicolor)* bran. *Journal of Food Biochemistry*, 38, 110-118.

Silva, C., Bates, R., & Deng, J., 1981. Influence of soaking and cooking upon the softening and eating quality of black beans *(Phaseolus vulgaris). Journal of Food Science*, 46, 1716-1720.

Singh, N., Kaur, M., Sandhu, K. S., & Guraya, H. S., 2004.Physicochemical, thermal, morphological and pasting properties of starches from some Indian black gram *(Phaseolus mungo L.)* cultivars.*Starch-Stärke*, 56, 535-544.

Sirivongpaisal, P., 2008. Structure and functional properties of starch and flour from bambarra groundnut.*Sonklanakarin Journal of Science and Technology*, 30, 51.

Slade, L., Levine, H., & Reid, D. S., 1991. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety.*Critical Reviews in Food Science & Nutrition*, 30, 115-360.

Swanevelder, C., 1998. Bambara–food for Africa.*National Department of Agriculture, Government Printer, Republic of South Africa*.

Szot, B., Horabik, J., & Rusinek, R., 2003. Physical properties characteristic of Polish and Canadian lentil seeds. *International Agrophysics*, 17, 123-129.

Tabilo-Munizaga, G., & Barbosa-Cánovas, G. V., 2005. Rheology for the food industry.*Journal of Food Engineering*, 67, 147-156.

Taiwo, K. A., Akanbi, C., & Ajibola, O. O., 1997. The effects of soaking and cooking time on the cooking properties of two cowpea varieties.*Journal of Food Engineering*, 33, 337-346.

Taylor, J. R. N., Schober, T. J., & Bean, S. R., 2006. Novel food and non-food uses for sorghum and millets: A review. *Journal of Cereal Science*, 44, 252-271.

Taylor, J. R. N., & Duodu, K. G., 2015. Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products.*Journal of the Science of Food andAgriculture*, 95,225-237.

Temba, M.C., Njobeh, P.B., Adebo, O.A., Olugbile, A.O., & Kayitesi, E., 2016. The role of compositing cereals with legumes to alleviate protein energy malnutrition in Africa. *International Journal of Food Science and Technology*, 51, 543–554.

Tharanathan, R., & Mahadevamma, S., 2003. Grain legumes-a boon to human nutrition.*Trends in Food Science & Technology*, 14, 507-518.

Trèche, C.M.S., 2001. Viscosity of gruels for infants: a comparison of measurement procedures. *International Journal of Food Sciences and Nutrition*, 52, 389-400.

Turnbull, C.M., Baxter, A.L., & Johnson, S.K., 2005. Water binding capacity and viscosity of Australian sweet lupin kernel fibre under *in vitro* conditions simulating the human upper gastro-intestinal tract.*International Journal of Food Sciences and Nutrition*, 56: 87-94.

Van Zuilichem, D. J., Van't Riet, K., & Stolp, W., 1986. An overview of near–infrared radiation processes from various agricultural products. In *Food Engineering and Process Applications: Vol. 1 Transport Phenomena*, 595–610. M. Le Maguer and P. Jelen, eds. London, U.K.: Elsevier Applied Science.

Venkatesh, M., & Raghavan, G., 2004. An overview of microwave processing and dielectric properties of agri-food materials. *Biosystems Engineering*, 88, 1-18.

Vilakati, N., Macintyre, U., Oelofse, A., & Taylor, J. R., 2015. Influence of micronization (infrared treatment) on the protein and functional quality of a ready-to-eat sorghum-

cowpea African porridge for young child-feeding. *LWT-Food Science and Technology*, 63, 1191-1198.

Wang, N., Daun, J. K., & Malcolmson, L. J., 2003. Relationship between physicochemical and cooking properties, and effects of cooking on antinutrients, of yellow field peas *(Pisum sativum)*. *Journal of the Science of Food and Agriculture*, 83, 1228-1237.

Wang, N., & Daun, J. K., 2005. Determination of cooking times of pulses using an automated Mattson cooker apparatus. *Journal of the Science of Food and Agriculture*, 85, 1631-1635.

Wang, N., Hatcher, D., Toews, R., & Gawalko, E., 2009. Influence of cooking and dehulling on nutritional composition of several varieties of lentils *(Lens culinaris)*. *LWT-Food Science and Technology*, 42, 842-848.

Waniska, R., & Myers, J. R., 2006. Analysis of Cooking and Consumer Preference Traits to Achieve Wider Impact from Bean/Cowpea CRSP Developed Bean Cultivars. *Research and Training Highlights*, 39.

Wiriyaumpaiwong, S., Soponronnarit, S., & Prachayawarakorn, S., 2004. Comparative study of heating processes for full-fat soybeans. *Journal of Food Engineering*, 65, 371-382.

Woolnough, J. W., Monro, J. A., Brennan, C. S. & Bird, A. R. 2008. Simulating human carbohydrate digestion *in vitro*: a review of methods and the need for standardisation. *International Journal of Food science & Technology*, 43, 2245-2256.

Xu, B. J., & Chang, S. K. C., 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents.*Journal of Food Science*, *72*, S159-S166.

Yagoub, A., & Abdalla, A. A., 2007. Effect of domestic processing methods on chemical composition, *in vitro* digestibility of protein and starch and functional properties of bambara groundnut *(Voandzeia subterranea)* seed. *Research Journal of Agriculture and Biological Sciences*, 3, 24-34.

Yoo, D., & Yoo, B., 2005. Rheology of rice starch-sucrose composites. *Starch-Stärke*, 57, 254-261.

Zhao, Y., 2000. The effects of treatment and micronization on the quality and cookability of lentils.Unpublished MSc thesis. Winnipeg, Manitoba: University of Manitoba, Department of Food Science.

Zheng, G., Fasina, O., Sosulski, F., & Tyler, R., 1998. Nitrogen solubility of cereals and legumes subjected to micronization. *Journal of Agricultural and Food Chemistry*, 46, 4150-4157.

8 PUBLICATION, PRESENTATIONS AND POSTERS FROM THIS RESEARCH

Ogundele, O. M., Minnaar, A., & Emmambux, M. N., 2017. Effects of micronisation and dehulling of pre-soaked bambara groundnut seeds on microstructure and functionality of the resulting flours. *Food chemistry,* 214**,** 655-663. Published.

Ogundele, O.M., Minnaar, A., & Emmambux, M.N., 2016. Effects of micronisation and dehulling of pre-soaked bambara groundnut seeds on the microstructure and functionality of the resulting flours. Poster presentation at IUFoST (International Union of Food Science and Technology) 18th World Congress of Food Science and Technology.2016 (21st- 25th, December 2016), Royal Dublin Society, Balls bridge, Dublin, Ireland.

Ogundele, O.M., Minnaar, A., & Emmambux, M.N., 2015. Effects of dehulling and micronisation of pre-soaked bambara groundnut seeds on functionality of the resulting flours. Oral presentation in 21th Biennial International SAAFoST Congress and Exhibition, Durban.

Ogundele, O.M., Minnaar, A., & Emmambux, M.N., 2014. Relating Microstructure and functionality of Micronised bambara pastes. Poster presentation at MSSA (Microscopy Society of Southern Africa) annual International Conference.2014 (2- 5th, December 2014), Protea Hotel Stellenbosch, South Africa.