Extruded sorghum and Bambara groundnuts: Influence of in-barrel moisture conditions on functional and nutritional characteristics

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DECLARATION

I declare that the dissertation, which I hereby submit for the degree MSc Food Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other university or institution of tertiary education.

Kara Muller

Date: 30/08/2016

DEDICATION

This dissertation is dedicated to the late Professor Amanda Minnaar. Her passion for food and people sparked in me a sense of responsibility which comes with knowledge, all inspired by a passionate and living God. Thank you Heavenly Daddy, for the privilege of exploring and discovering your vast yet detailed, creative and intelligent creation. This work and all lessons learnt in the process testifies of Your heart for all people. I pour it all at Your feet.

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ABSTRACT

Extruded sorghum and bambara groundnuts: Influence of in-barrel moisture conditions on functional and nutritional characteristics

By

Kara Muller

Supervisor: Prof. A. Minnaar (deceased)

Co-supervisor: Prof. J.R.N. Taylor (supervisor from October 2015)

Co-supervisor: Dr J. Kruger

Protein-energy malnutrition (PEM) and micronutrient deficiencies remain burdens among children in Africa due to monotonous cereal-based diets. Compositing with local pulses such as bambara groundnuts can be a solution. Rapidly urbanising communities in Africa, however, demand convenience-type products. To address these needs, the nutritional, physical and functional properties of extrudates of decorticated sorghum and whole grain bambara groundnut flours extruded separately and as a 50:50 composite using a twin screw extrusion cooker was investigated. The effect of two in-barrel moisture conditions, low (24%) and high (40%), were studied. Compositing sorghum with bambara groundnuts increased crude fibre (190%), protein (54%) fat (36%) and mineral (118%) contents. Zinc, calcium and magnesium contents increased by 32, 52, and 11%, respectively with bambara groundnut inclusion. *In vitro* protein digestibility (IVPD) and nitrogen solubility index (NSI) increased substantially with bambara groundnut inclusion because bambara groundnuts' soluble globulin-type proteins are more digestible than sorghum kafirins. Extrusion cooking caused an increase in IVPD and decrease in NSI. Hot, moist conditions in extrusion cooking probably caused the denaturation of proteins which exposed hydrophobic sites of the protein molecule and exposed more sites for proteolytic attack. Extrusion cooking at high moisture yielded a slightly higher IVPD in composite extrudates and higher NSI in bambara groundnut extrudates, as shear and heat generation would be less severe, compared to low moisture. Phytate content was reduced (12-35%) in the composite and bambara groundnut extrudates after extrusion cooking at both extrusion moistures. Inositol hexaphosphate was possibly hydrolysed to penta- and tetraphosphates. Improved Caco-2 cell zinc uptake (80-84%) was achieved through both extrusion moistures in sorghum extrudates only, probably due to lower phytate x calcium/zinc ratios in sorghum when compared to the composite and bambara groundnut flours. Bambara groundnut inclusion reduced Caco-2 cell zinc uptake due to an increase in phytate content.

High extrusion moisture and bambara groundnut inclusion decreased extrudate expansion in sorghum and the composite, probably due to lower viscosity in the extruder barrel and dilution of starch, respectively. High moisture conditions yielded higher overall water absorption index (WAI), lower overall water solubility index (WSI) and higher peak, trough and final paste viscosities, probably due to less starch degradation. Bambara groundnut inclusion caused increased WSI, probably due to its soluble proteins. It also caused lower peak, trough and final paste viscosities, possibly due to starch dilution.

A 50 g (db) serving of sorghum-bambara groundnut composite extrudate can contribute 81% of the daily protein requirement for children aged 2-5 years. Improved IVPD, WAI and paste viscosities for composite extruded at low moisture suggests its application as a nutrient-dense instant porridge. Extrusion cooking of the composite at low moisture also maintains high expansion which can produce a nutritious expanded snack. Sorghum-bambara groundnut composite extruded at low moisture can produce convenience-type products with improved mineral content and excellent protein content and digestibility to address PEM.

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1 INTRODUCTION

Sub-Saharan Africa remains the region with the highest prevalence of undernutrition (UN, 2015). Undernutrition hugely impacts on children under 5 years, each year causing about 45% of deaths in children worldwide (WHO, 2016). Protein-energy malnutrition (PEM) and deficiencies of micronutrients such as iron, zinc, iodine and vitamin A are the major forms of malnutrition prevalent among children and manifests in the forms of stunting, wasting and increased disease burden which often lead to mortality (Black et al., 2008). A predominance of poor diets and infectious disease in developing communities go hand-in-hand as causes of these mortalities (Müller & Krawinkel, 2005).

Starchy cereals, roots and tubers are the staple foods of people in sub-Saharan Africa (WHO, 2014). The monotony of starch possibly explains the lack of protein and micronutrients in the diets of children, leading to malnutrition. Based on production in 2014, sorghum is quantitatively the second most important cereal in Africa after maize (FAOSTAT3, 2014). One of the key values of sorghum is that it is able to grow in poor soils and drought stricken areas where subsistence farmers do not have irrigation systems in place (Dalal et al., 2012). Nutritionally, however, sorghum is especially poor in the essential (indispensable) amino acid, lysine (Serna-Saldivar & Rooney, 1995). Furthermore, sorghum protein digestibility is largely reduced on wet cooking to prepare traditional foods (Hamaker et al., 1986).

Food-based strategies, such as dietary diversification, have been successful in addressing malnutrition (Tontisirin et al., 2002). As animal proteins are costly and scarce in developing communities (Iqbal et al., 2006), incorporation of these excellent protein sources into the diets of children to prevent PEM, is sometimes not feasible. The development of cereal and legume composite foods is a good dietary diversification strategy to improve the protein quality of traditional starchy foods. Compositing has proven to be successful in the production of protein-rich weaning foods where sorghum has been composited with various legume seeds (Asma et al., 2006).

Bambara groundnut is a drought-tolerant and nutritious indigenous African grain legume (pulse) with high protein content, but is underutilised (Jideani & Diedericks, 2014). Due to the bambara plants' nitrogen fixation abilities, it is applied in intercropping and crop rotation with other grains such as sorghum (Dakora & Keya, 1997). The combination of being tolerant to low soil fertility and low rainfall and being rich in high-quality protein (Azam-Ali et al., 2001), makes bambara groundnuts a potential solution to address poor diets, recurring drought and soil degradation in sub-Saharan Africa (Solomon et al., 2016). However, bambara groundnuts remain mostly utilised by subsistence farmers (Muhammad, 2014).

The presence of anti-nutritional compounds in sorghum and bambara groundnuts presents limitations to the nutritional attributes of these grains. The presence of phytate and polyphenolic compounds in plant foods inhibits the absorption of minerals in plant-based diets (Hunt, 2003). Another challenge in the application of these grains is its tedious preparation, especially of bambara groundnuts (Jideani & Diedericks, 2014).The rapidly urbanising communities of Africa (UN, 2014) leads to fast-paced lifestyles where long food preparation times are burdensome.

Extrusion cooking is a food processing technology which can be applied to produce a variety of convenience-type products from a large diversity of raw materials (Guy, 2001). Extrusion cooking is a continuous manufacturing process which applies high heat, pressure and friction to break down raw foods to a cooked and pre-gelatinised form (Fellows, 2009). It is also effective in destroying anti-nutritional compounds and enhances the digestibility of plant proteins (Abd El-Hady & Habiba, 2003). The application of different processing conditions (Chessari & Sellahewa, 2001) and raw materials (Guy, 2001) in extrusion cooking yields products with varying nutritional, physical and functional properties. Regarding processing conditions, extruder in-barrel moisture content is considered as having the greatest impact on product properties (Bhattacharya, 2012).

Extrusion cooking could, therefore, be applied to produce convenience-type foods from African grains such as sorghum and bambara groundnuts. Compositing sorghum with bambara groundnuts could help alleviate protein and mineral deficiencies in the starch-based diets of children. Extrusion of composites could also hold the benefits of improved protein quality, whilst also addressing the issue of low mineral availability.

This study is the first of its kind where high (40%) and low (24%) extrusion moisture conditions are investigated for processing a composite of sorghum with bambara groundnuts. The aim is to establish the effects of compositing and extrusion moisture on product nutritional, physical and functional properties. This will indicate which conditions could yield nutritionally beneficial products and how they could be applied as convenience-type nutritious foods for children aged 2 to 5 years.

2 LITERATURE REVIEW

In this review, the protein and mineral requirements of children aged 2-5 years are briefly described. In light of these needs, the nutritional composition with a specific focus on protein quality and mineral bioaccessibility of sorghum and bambara groundnuts are reviewed. The principles of extrusion cooking, especially the effects of legume flour and of extrusion moisture levels on the nutritional, physical and functional qualities of extrudates, are also reviewed.

2.1 Protein and mineral requirements of children

Malnutrition is a continuous burden for developing countries (UNICEF, 2015) with the two major problems being PEM and micronutrient deficiencies (Müller & Krawinkel, 2005). Poor nutrition causes 45% of deaths in children under five years of age across the world (WHO, 2016). These deaths are due to the increased susceptibility to diseases (Dewey & Mayers, 2011) together with impaired physical and cognitive development (Grantham-McGregor & Ani, 2001), as the major consequences of malnutrition.

Growth in children involves increases in height and mass, together with development and maturation of body function (WHO/FAO/UNU, 2007). Growth requires sufficient amounts of certain amino acids, more than are required for the resting metabolic state, for deposition of extracellular proteins, DNA, RNA, cell membranes, creatine and haem to take place. These processes enable the formation of new cells and tissue to enable height and mass increases (WHO/FAO/UNU, 2007). The protein requirements (Table 2.1) for children have been defined as "the minimum intake that will allow nitrogen equilibrium at an appropriate body composition during energy balance at moderate physical activity, plus the needs associated with the deposition of tissues consistent with good health" (WHO/FAO/UNU, 2007).

Regarding micronutrients, deficiencies of iron, zinc and vitamin A are most prevalent in developing countries (Müller & Krawinkel, 2005). Micronutrient deficiencies often coexist with PEM due to the over consumption of cereals and tubers, which are generally poor in micronutrients, and due to the scarcity of animal products in the diet, which are rich in easily digestible proteins and available minerals (Makonnen et al., 2003). Iron deficiency can adversely affect cognitive performance, growth of infants and children and resistance to infections (WHO/UNICEF/UNU, 2001). Deficiencies in zinc pose similar problems together with delayed sexual and bone maturation and skin lesions (WHO/FAO, 2004).

There is a large variation in iron and zinc bioavailability from plant-based diets compared to diets including animal products. Due to this variation, different recommended nutrient intakes (RNI's) have been calculated for these minerals to take bioavailability into account. The iron and zinc RNI at low bioavailability (Table 2.1) is more than twice the RNI at high bioavailability (WHO/FAO, 2004).

Table 2.1 Safe level of protein intake and recommended nutrient intakes of iron and zinc at low bioavailability for children aged 2-5 years

	Age (years)		
	2	**3**	**4 and 5**
Protein intake[1] (g protein/day)	11.7	12.9	16.7
Lysine requirements[1] (mg/g protein requirement)	52	48	48
Iron intake[2] (mg/day)	11.6	11.6	12.6
Zinc intake[2] (mg/day)	8.3	8.3	9.6

[1]From WHO/FAO/UNU (2007)
[2]From WHO/FAO (2004)

2.2. Nutritional composition and quality of sorghum and bambara groundnuts

2.2.1 Nutrient composition of sorghum and bambara groundnuts

Sorghum grain consists mainly of starch (68-77%) (Neucere & Sumrell, 1980) of which the main starch macromolecule is amylopectin. Starch amylose contents range from 21–30% (Beta & Corke, 2001). Protein is the second most abundant chemical component ranging from 7-12% (Jones & Beckwith, 1970). The protein content of sorghum is generally slightly higher than that of other important cereals such as maize, rice and millets (Adeyeye & Ajewole, 1992). The main protein fraction in sorghum is kafirin, the sorghum prolamin storage protein (Taylor & Schüssler, 1986).

The main chemical component of bambara groundnuts is also starch (50-65%) (Igbedioh et al., 1994) but it has a larger proportion of amylose (37%) (Ashogbon, 2014) than sorghum starch. The amylose content of bambara groundnuts is in line with the amylose contents of other pulses which are generally higher (35-46%) than cereals (Rao, 1976). Bambara groundnuts are also richer in protein (17-25%) compared to sorghum, with major protein fractions being albumins and globulins (Poulter, 1981). The protein content of bambara groundnuts is similar to that of other common pulse-like legumes such as chickpeas, cowpeas, lentils and green peas (Iqbal et al., 2006). Oligosaccharides of the raffinose sugar family are predominant in most legumes, of which stachyose seems to be more abundant (0.75-1 g/100 g) in bambara groundnuts (Apata, 2008). Oligosaccharides in legumes can cause undesirable flatulence after consumption (Salunkhe & Kadam, 1989). The benefits of oligosaccharides, however, include the production of lactic acid and short chain fatty acids (SCFA) in the intestine as products of fermentation by anaerobic bacteria (Salunkhe & Kadam, 1989). SCFA provide many benefits to human health such as suppression of pathogen growth in the gut, influencing intestinal motility and possible inhibition of cholesterol synthesis (Roberfroid et al., 2010).

Table 2.2 shows that the proximate composition of bambara groundnuts is superior to sorghum in terms of protein, fat and minerals. Due to bambara groundnuts' high protein and mineral contents, it is used in composites with cereals and even tubers to improve the nutritional qualities of traditional meals. Cereals and tubers which are composited with bambara groundnuts for traditional foods include maize for traditional African maize-based foods such as 'ogi', a starchy gruel (Mbata et al., 2009), pearl millet for the formulation of 'agidi', a stiff gel, (Zakari et al., 2010); malted sorghum and fermented sweet potato for porridge (Nnam, 2001) and fermented cassava for the production of 'fufu' (Olapade et al., 2014). Bambara groundnuts have also been investigated in composites in products such as biscuits (Abu-Salem & Abou-Arab, 2011) and bread (Alozie et al., 2009). Extrusion cooking has been applied to produce white yam and bambara groundnut expanded extrudates (Oluwole & Olapade, 2011), sorghum malt and bambara groundnut based extrudates (Jiddere & Filli, 2015) and pearl millet and bambara groundnut based fura (Filli et al., 2013).

Table 2.2 Proximate compositions (g/100 g) of whole grain sorghum and bambara groundnuts

	Moisture	Ash (minerals)	Protein	Fat	Crude fibre	Starch
Sorghum[1]	12.0	1.6	10.9	3.2	2.3	73.0
Bambara groundnuts[2]	12.5	3.8	22.5	7.0	1.7	52.5

[1] From Serna-Saldivar and Rooney (1995)
[2] From Falade and Nwajei (2015)

To render sorghum flour more palatable, some form of decortication (debranning) is generally applied before further milling or further use (Taylor, 2003). This can be achieved industrially using dehullers, or traditionally using a mortar and pestle (Munck, 1995). The various anatomical components of any cereal grain differ in chemical composition. Decortication, which involves removal of the bran and germ (Taylor, 2003) will, therefore, alter the chemical composition of sorghum flour when compared to the whole grain. Removal of the bran and germ can cause a loss in protein, ash, fat (lipids) and dietary fibre (Serna-Saldivar & Rooney, 1995). A reduction in tannin content can also be expected with the removal of sorghum bran (Chibber et al., 1978) if the sorghum variety is a tannin type.

2.2.2 Protein quality

Protein quality can be defined by the digestibility and amino acid composition of the proteins in the considered food (FAO/WHO/UNU, 1985). Therefore, even though a food source might have a high protein content, the available amino acid composition and protein digestibility of the proteins present would dictate whether proteins are of high quality to the consumer.

The amino acid composition of sorghum falls short in the amount of lysine (up to 23.3 mg/g protein) required from ingested proteins for children of ages 2-5 years (Elkin et al., 1996) as determined by WHO/FAO/UNU (2007) (48-52 mg/g protein required). Even though cereals are generally low in the indispensable amino acid lysine, it seems that sorghum naturally has a greater deficiency in this amino acid (Serna-Saldivar & Rooney, 1995). Sorghum proteins are, however, relatively high in the sulphur-containing amino acids cysteine and methionine (up to 22.8 mg methionine/g protein) (Elkin et al., 1996).

Bambara groundnut's lysine content, in contrast, is remarkably higher than sorghum (up to 80.3 mg/g protein) although its essential amino acid methionine content is generally lower (6.4 mg/g protein) (Yao et al., 2015). The amino acid profile of the two grains can clearly complement each other. It is essential, however, to consider the ability of these amino acids to be absorbed after consumption.

Protein digestibility indicates the proportion of a food protein ingested which is digested and absorbed in the gastrointestinal tract (WHO/FAO/UNU, 2007). It is essentially a measure of the availability of amino acids for absorption after proteolysis (Hsu et al., 1977). The *in vitro* protein digestibility (IVPD) of uncooked decorticated sorghum has been found to be between 72 and 86% (Axtell et al., 1981; Hamaker et al., 1994). The IVPD of sorghum has, however, been shown to decrease by 16-41% (Chibber et al., 1978; Hamaker et al., 1986; Oria et al., 1995) after processing of sorghum flour by wet cooking. This decrease in IVPD after cooking has been found to be unique to sorghum grain (Hamaker, 1986) and does not occur to any significant extent in other cereals (Mertz et al., 1984). Duodu, Taylor, Belton and Hamaker (2003) summarised many of the factors which can affect sorghum protein digestibility. The low IVPD of sorghum can to a great extent be attributed to its prolamin protein (kafirin). It has been proposed that disulphide bonds form between cysteine-rich β- and γ-type kafirins at the periphery of sorghum protein bodies (Hamaker et al., 1994), the organelles of kafirin storage, possibly leading to a rigid β-sheet conformational structure (Emmambux & Taylor, 2009). It has been further proposed that disulphide bonding of protein at the protein body surface inhibits proteolytic attack of the more digestible α-kafirins located in the centre of protein bodies, therefore hindering the digestibility of the major kafirin sub-group, the α-kafirins (Oria et al., 1995).

The IVPD of bambara groundnuts has been reported to be approximately 77% (Nwokolo, 1987). Although the protein digestibility of raw bambara groundnuts might seem lower than that of raw sorghum, thermal processing of legumes have been found to improve protein digestibility (Liener, 1976), as opposed to sorghum. Extrusion cooking was also found to cause a great increase in the IVPD of various legumes (Abd El-Hady & Habiba, 2003). The improvement of IVPD in legumes after heat treatment, as opposed to the decrease of IVPD in sorghum, is due to two factors. Firstly, anti-nutritional factors which cause low IVPD, in particular, enzyme inhibitors such as trypsin and chymotrypsin inhibitors present in legumes, are heat labile and therefore destroyed after heat treatment (Alonso et al., 2000). Rheman and Shah (2005) showed how the protein digestibility of various legumes improved with a decrease in anti-nutritional factors through boiling. This was also found by Shimelis and Rakshit (2007). Additionally, more hydrophilic sites of the globulin storage proteins of legumes are exposed after heat treatment due to the unfolding of protein units during denaturation. Hence, hydrophilic sites susceptible to proteolytic attack are exposed, which renders the proteins more readily digested than in their native state (Nielsen et al., 1988).

2.2.3 Mineral composition and quality

Cereal-legume based food composites are regarded as a valuable food diversification strategy to help maintain a healthy intake of micronutrients in deficient diets (Tontisirin et al., 2002). Table 2.3 shows the mineral contents of sorghum and bambara groundnuts. Bambara groundnuts contain higher levels of calcium and iron than sorghum. Further, decortication of sorghum will cause a reduction in the amount of minerals in the resulting sorghum flour as the minerals are concentrated in the pericarp and aleurone layer (Serna-Saldivar & Rooney, 1995).

Table 2.3 Mineral composition (mg/100 g dry basis) of whole grain sorghum and bambara groundnuts

	Fe	Zn	Ca	Mg	P
Sorghum[1]	3.0	1.7	13	165	289
Bambara groundnuts[2]	8.8	1.9	30	136	336

[1] From USDA (2015)
[2] From Yao et al. (2015)

Gibson and Hotz (2001) stated that although a diet based on unrefined cereals and legumes can supply a fair amount of minerals, the bioavailability of minerals is not adequate to combat mineral deficiencies. This is due to high levels of the anti-nutrients phytic acid and polyphenols present in cereals and legumes. These form insoluble complexes with minerals, thereby inhibiting their absorption. Other intrinsic components such as oxalic acid, proteins, fibre and lignin also interact with minerals to affect their bioavailability (Sandberg, 2002). As in the case of proteins, it is important to consider the bioavailability of minerals in plant foodstuffs according to interaction with anti-nutritional factors.

Mineral dialysability and uptake are ways of determining mineral bioaccessibility, representing different levels of human absorption as described in a review by Fairweather-Tait et al. (2005). The mineral dialysability and Caco-2 cell uptake of iron from sorghum have been found to be 6.7% and 0.82%, respectively (Kruger et al., 2013). In the same study, the mineral dialysability and Caco-2 cell uptake of zinc were 7% and 1.21%, respectively. High iron and zinc bioaccessibilities are regarded at dialysability levels of 15% and 50%, respectively (Kruger et al., 2013). The bioaccessibility of these important minerals can, therefore, be regarded as low in sorghum. Strategies to improve bioavailability should, therefore, be considered to maximise the uptake of minerals present. Viadel, Barberá and Farré (2006) showed that the calcium, iron and zinc uptake by Caco-2 cells from different legume species varied greatly, depending on the unique chemical composition of each legume species. The uptake of these minerals was improved when the legumes were processed by wet cooking. Iron and zinc uptake values for raw legumes ranged between 0.46-11.94% and 1.02-6.70%, respectively. The iron and zinc uptake of white beans and chickpeas in this study far exceeded the iron and zinc uptake of sorghum found by Kruger et al. (2013). The bioaccessibility of iron in raw dehulled cowpeas was also found to be higher than that of raw decorticated sorghum (Vilakati et al., 2016).

2.2.4 Vitamin composition

Sorghum is considered a good source of B-vitamins, which are generally concentrated in the aleurone layer and germ (Serna-Saldivar & Rooney, 1995). Sorghum is, however, not a source of vitamin B-12 Vitamin E is also present in the germ of sorghum (Serna-Saldivar & Rooney, 1995). Food legumes are good sources of the B vitamins thiamin, riboflavin and niacin (Salunkhe & Kadam, 1989). Sorghum appears to be richer in niacin, however, whereas bambara groundnuts have superior thiamin, biotin and folacin contents (Table 2.4). Reliable data on the vitamin E content of Bambara groundnuts are lacking

Table 2.4 B-vitamin composition (mg/100 g dry basis) of whole grain sorghum and bambara groundnuts

	Thiamin	Riboflavin	Niacin	Biotin	Folacin
Sorghum	0.35[1]	0.16[1]	4.201[1]	0.02[2]	0.02[1]
Bambara groundnut[2]	0.47[3]	0.16[3]	1.88[3]	0.10[3]	0.16[3]

[1]From Ochanda, Onyango, Mwasaru, Ochieng and Mathooko (2010)
[2]From Misir and Blair (1988)
[3]From Fadahunsi (2009)

2.3 Anti-nutritional factors in sorghum and bambara groundnuts

2.3.1 Phenolic compounds

Phenolic compounds are the largest group of secondary metabolites found in plant foods (Kroll et al., 2003). Although phenolic compounds are seen as health-promoting, they also function as anti-nutrients. They can be divided into three major categories: phenolic acids, flavonoids and tannins (Serna-Saldivar & Rooney, 1995).

Phenolic compounds are located in the pericarp, testa, aleurone layer, and endosperm of sorghum grain (Hahn et al., 1984) but can be significantly reduced through decortication (Awika et al., 2005a). All sorghums contain phenolic acids of which ferulic, *p*-coumaric, and cinnamic acids are the major types (Dykes & Rooney, 2006). Anthocyanins are the major class of flavonoids in sorghum with the content being closely related to pericarp colour; black sorghum grains containing the highest amounts (Awika et al., 2005b). Other types of flavonoids present in sorghums depend greatly on their variety and colour (Dykes & Rooney, 2006). Only certain sorghum types contain condensed tannins (Hahn et al., 1984). High tannin sorghums and black non-tannin sorghums generally contain the highest levels of phenolics (Dykes &

Rooney, 2007). A great difference in phenolic contents between tannin and non-tannin varieties have been reported as 19.7-24.5 and 0.27-0.53 g catechin equivalents (CE)/100 g sorghum, respectively (Dlamini et al., 2007).

Bambara groundnuts also contain polyphenols in the seed coat and have been reported to contain 0.71 g CE/100 g bambara groundnuts (Yao et al., 2015). This is similar to values reported for other legumes, which range broadly between 0.03 and 1.7 g CE/100 g (Bravo, 1998). The phenolic compounds in bambara groundnuts are less studied and defined than those in sorghum. Condensed tannins have, however, been widely measured and range from 0.4 to 1.5 g CE/100 g bambara groundnut, depending on the seed coat colour (Nti, 2009). The majority of phenolic compounds, however, have been identified as flavonoids (Nyau et al., 2015).

Polyphenols inhibit the absorption of non-haem iron which is found in plants. Non-haem iron is not protected from anti-nutritional factors by a porphyrin ring, as in haem-iron (Andjelković et al., 2006). It is proposed that polyphenols form complexes with minerals through their hydroxyl groups (Khokhar & Owusu Apenten, 2003), therefore acting as metal chelators (Andjelković et al., 2006). Condensed tannins are the only type of phenolics which complex strongly with proteins and hence adversely affect the biological function (enzymes) or digestibility of proteins (Emmambux & Taylor, 2003). Tannins have a high affinity for proteins and bond to their structures through covalent- or hydrogen bonding or through hydrophobic association (Oh et al., 1980).

2.3.3 Phytate

Phytate is the salt of *myo*-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate ($InsP_6$), popularly referred to as its negatively charged molecule, phytic acid (Blaabjerg et al., 2010). It is the main form in which phosphorus is stored in cereals and legume grains (Blaabjerg et al., 2010).

The negatively charged phytic acid has a strong affinity to bind to divalent metal cations. It acts as a ligand, binding with minerals such as iron, zinc and calcium to form the stable phytin molecule, as shown in Figure 2.1 (Lopez et al., 2002). Minerals bound in this way are unavailable for absorption as they are not in their ionic form. Phytic acid also has the ability to form complexes with proteins. Depending on protein type and pH of the system, proteins can be positively charged, enabling interaction with phytic acid (Oatway et al., 2001), therefore inhibiting key digestive enzymes such as trypsin, pepsin and amylase (Urbano et al., 2000). The hydrolysis products of $InsP_6$ include *myo*-inositol pentakis- ($InsP_5$), tetrakis- ($InsP_4$), tris-

($InsP_3$), bis- ($InsP_2$) and monophosphates ($InsP_1$) (Blaabjerg et al., 2010). These hydrolysis products can also form complexes with minerals and proteins, though the binding strength of lower inositol molecules with minerals (Persson et al., 1998) and proteins (Yu et al., 2012) is weaker.

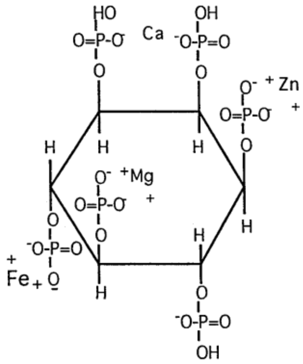


Figure 2.1 Structure of the phytin molecule (Deluca & Baker, 1999)

Phytate in cereals such as sorghum is mostly stored in the aleurone layer and can, therefore, be largely reduced by decortication. (Serna-Saldivar & Rooney, 1995). In a study of 45 sorghum lines, phytate contents varied between 0.47 and 3.53 g/100 g, depending on the genetic and environmental factors (Kayodé et al., 2006). The phytate contents of other common cereals such as barley, maize, millets, oats, rice and wheat are similar (Oatway et al., 2001). In dicotyledonous seeds such as bambara groundnuts, on the other hand, phytate is found in globoid crystals in the cotyledons (Urbano et al., 2000). Notwithstanding this, the phytate content of bambara groundnuts also seems to vary greatly and has been reported to be between 0.29 and 4.17 g/100g (Igbedioh et al., 1994; Fasoyiro et al., 2006). The phytate content of bambara groundnuts seems to be somewhat higher than cowpea, soybean and groundnut (1.28-1.73 g/100 g), but similar to African yam bean, lima bean and pigeon pea (Fasoyiro et al., 2006).

2.3.4 Trypsin inhibitors and lectins

Trypsin inhibitors and lectins are protein-type anti-nutritional factors. Lectins are sugar-binding proteins which are resistant to breakdown by digestive enzymes (He et al., 2015). Lectins' high affinity for carbohydrates can, at high dosages, cause the binding of these substances to epithelial cells in the digestive tract (He et al., 2015). Subsequently, intestinal permeability can be changed which is disadvantageous for the absorption of necessary nutrients. It is still unclear exactly which lectins are toxic to the extent of causing symptoms such as nausea, bloating, vomiting, diarrhoea and even death (Vasconcelos & Oliveira, 2004). Some lectins also show the ability to agglutinate red blood cells and are regarded as haemagglutinins (Ayyagari et al., 1989). Lectins are often quantified using blood cell agglutination techniques. There is no specific relationship, however, between haemagglutinating activity and toxicity of lectins (Vasconcelos & Oliveira, 2004).

Trypsin inhibitors, a class of protease inhibitors, inhibit the activity of protein digestive enzymes, particularly trypsin (Rackis et al., 1985). Apart from an inability to subsequently absorb amino acids in the gut, the enzyme inhibition has even been found to cause pancreatic hypertrophy, affecting pancreatic functioning (Rackis et al., 1985).

Only haemagglutinating activity measurements of sorghum and bambara groundnuts, to the knowledge of the author, are reported in literature to serve as an indication of lectins. As with other cereals, sorghum varieties showed no haemagglutinating activity (Ayyagari et al., 1989). Bambara groundnuts, on the other hand, showed haemagglutinating activity of 2.6 x 10^{-6} HU/kg seed, which was lower than in cowpeas (10.4 x 10^{-6} HU/kg seed) (Grant et al., 1995). Sorghum trypsin inhibitors are not of great concern as they are easily inactivated by heat. The trypsin inhibitor content of bambara groundnuts has been found to be reducedto some extent (16 – 37 % reduction) through fermentation, germination and roasting (Ijarotimi & Esho, 2009). Bambara groundnut trypsin inhibitor content (19.08 TIU/mg sample) is lower than in lima beans and pigeon peas (20.4-29.6 TIU/mg), but slightly higher than cowpea and soybean (12.5-17.1 TIU/mg) (Fasoyiro et al., 2006).

2.4 Extrusion cooking technology

2.4.1 Principles

'Extrusion' refers to a process by which liquid to semi-liquid products are forced through a die opening of a desired shape and size (Kazemzadeh, 2012). Different extrusion technologies operate on the same principle, but have developed over many years to serve many additional functions in food preparation. These functions include conveying, mixing, shearing, separation, heating or cooling, co-extrusion, venting volatiles and moisture, flavour generation, encapsulation and sterilisation (Guy, 2001). Depending on the desired end-product, extruders are manufactured and applied to make use of specific functions. Cold extrusion has, for instance, been identified as extrusion where food is kept below temperatures of 100 °C (Fellows, 2009). Extrusion cooking falls under the category of High Temperature Short Time (HTST) processing (temperature conditions above 100 °C) together with the application of pressure and shear (Mościcki & Van Zuilichem, 2011). Extrusion cooking is applied in the production of ready-to-eat cereals, meat-like products from high-protein flours and nutritious food mixtures for infant feeding (Singh et al., 2007).

Mixing, cooking, kneading, shearing, shaping and forming are the operations combined in extrusion cooking to continuously break down raw foods to a cooked and pre-digested form (Fellows, 2009). What makes extrusion cooking unique compared to other HTST processes are the shear forces applied, which break covalent bonds in biopolymers. (Carvalho & Mitchell, 2000).

Many chemical reactions such as gelatinisation of starch, denaturation of proteins and Maillard reactions also take place during extrusion cooking (Mościcki & Van Zuilichem, 2011). All these reactions change the physical and chemical properties of the processed materials, thereby affecting the product's functional and nutritional properties. The outcome of extrusion cooking is shelf-stable, ready-to-eat and easily packaged products (Kazemzadeh, 2012). Extrusion cooking also reduces the number of microorganisms and inactivates endogenous enzymes (Fellows, 2009). The low water activity in the end products of extrusion cooking (7-10%), which is often achieved by drying of the extruded products is, however, the main method of preservation (Mościcki, 2011). It is maintained during storage in suitable packaging materials.

Extrusion cooking has many benefits and hence is a very popular processing method. Benefits include versatility in end products, low processing costs with high productivity and the production of almost no waste products such as effluents (Guy, 2001). Extrusion cooking also offers the potential to use raw materials previously regarded as economically unimportant or as waste to produce quality products. The ability to utilise waste is due to the various ways extrusion offers to manipulate different food matrices through effective control of the process parameters (Reimerdes, 1990).

There are many key control points throughout the extrusion cooking process which have an effect on the end product quality. The extrusion cooking process with control points related to end product quality is shown in Figure 2.2. The effects of some of these control points will be considered further, focusing on material formulation and in-barrel moisture levels which relate to water injection rate shown in Figure 2.2.

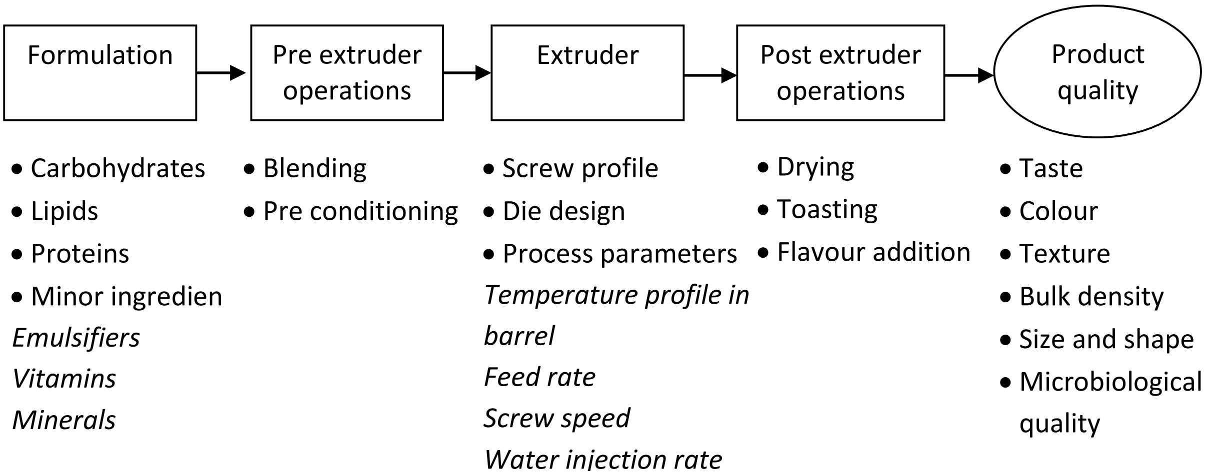


Figure 2.2 Key control points in meeting extrusion product requirements (Chessari & Sellahewa, 2001)

To understand the role of raw materials in product quality, an understanding of extrusion cookers and the extrusion cooking process is necessary. Among many different options to choose from in extrusion cooking, is the application of either one or two screws in the extruder barrel, referred to as single or twin screw extrusion cookers (Riaz, 2001). In short, twin screw extruders have greater flexibility in terms of raw material size and nature (viscous, oily, wet or sticky) and have greater control of parameters to achieve the desired end product (Fellows, 2009). Single screw extruders show low efficiency when a multi-component mixture is used as raw ingredient (Mościcki & Van Zuilichem, 2011). The only disadvantages of twin screw extrusion cookers are that they are of a more complicated design and have higher initial costs (Mościcki & Van Zuilichem, 2011). A schematic representation of a single screw extrusion cooker is shown in Figure 2.3. The basic parts of single and twin screw extrusion cookers are very similar, except for the screws (Riaz, 2001).

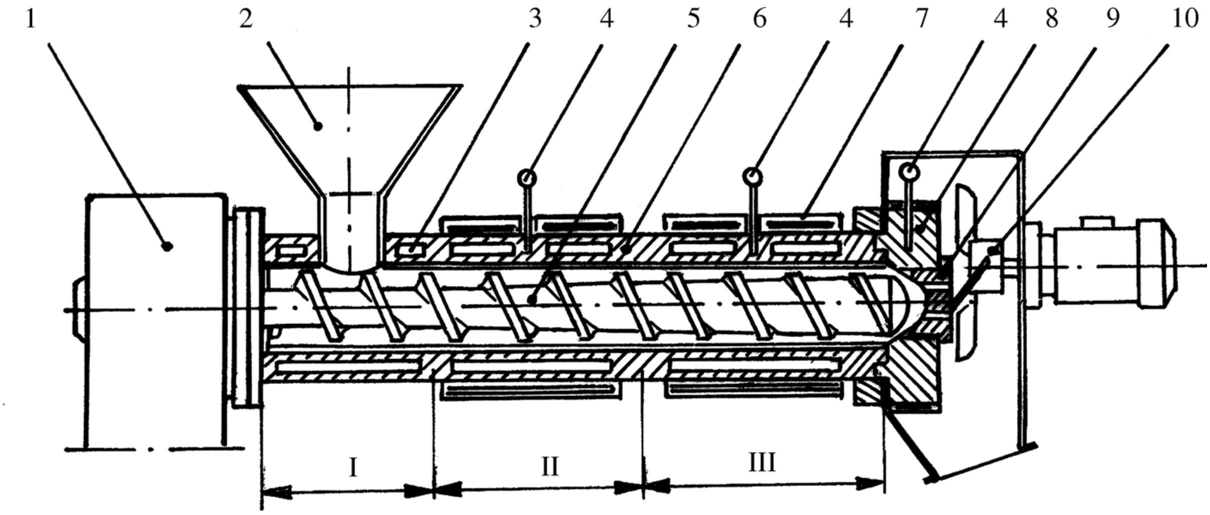


Figure 2.3 A cross-section of a single-screw food extruder: 1 – motor, 2 – feeder, 3 – cooling jacket, 4 – thermocouple, 5 – screw, 6 – barrel, 7 – heating jacket, 8 – head, 9 – dies, 10 – cutter, I - transport section, II – compression section, III –melting and plasticizing section (Mościcki & Van Zuilichem, 2011)

Food meal or flour flows from the feeder (2) into the barrel (6) and is conveyed by the screws (5) through the barrel to the dies in the head section (8). As the semi-liquid extrudate exits the dies (9), the cutter blades (10) cut the cooked product into desired sizes. The screws are suspended only from the motor end of the barrel (1) and rest on the product at the exit end, which results in great stress and pressure (Riaz, 2001). The food melt is conveyed through three identified sections: the transport (I), compression (II) and plasticizing sections (III). In the transport (conveying) section, feed is introduced to the extrusion cooker at a constant speed into the flights of a rapidly rotating screw (Yacu, 2012).

Cooking in the extrusion cooker starts mainly in the compression section and intensifies in the plasticizing section. The change in chemical structure in the material related to cooking is due to three sources of energy: conductive energy through heating of the barrel; convective energy through steam within the barrel from incorporated moisture, reaching high temperatures and pressures; conversion energy when the movement of the screws in the confined space of the barrel causes shear and produces pressure (Kazemzadeh, 2012). Exposure of the food material to these energies brings the food material to its melting or plasticating point (Mościcki & Van Zuilichem, 2011), after which it is forced through one or more die openings. As the food material is forced out under pressure through the die, it expands to the final shape and cools rapidly to retain its structure as moisture is flashed off as steam (Fellows, 2009).

Extruder constants which affect product quality, include the extruder screw configuration, length to diameter ratio of the extrusion cooker and design of the cutter at the end of the die

(Chessari & Sellahewa, 2001) (Fig 2.2). They also include the die design, which determines the product size and shape, the pressure drop across the die and the barrel fill, therefore influencing the energy generated in the extruder. Once the extrusion cooker is running and feed is introduced, flexible process variables can have an impact on end product quality (Table 2.5).

Table 2.5 Extrusion cooking process variables and their function in the process (Chessari & Sellahewa, 2001; Yacu, 2012)

Process variable	Effect
Screw speed	Affects the residence time of product in the barrel, amount of shear and frictional energy generated, barrel fill which affects melt viscosity.
In-barrel moisture content	Controls the frictional energy generated by affecting the melt viscosity.
Feed rate	Controls the amount of feed in the barrel which in turn affects residence time and pressure in the barrel.
Barrel temperature	Determines product temperature, affecting the degree of cook and melt viscosity.

Moisture levels in the barrel are regarded as the most important variable as they significantly impact all the energies involved in cooking the raw materials (Bhattacharya, 2012). Concerning moisture as processing parameter, different extrusion cooking applications have been identified on the basis of the moisture levels introduced into the extrusion cooking system. High moisture extrusion cooking is defined as extrusion cooking which takes place at in-barrel moisture contents of 40% and above and is mainly applied in twin screw extruders (Akdogan, 1999), whereas low moisture extrusion cooking takes place at in-barrel moisture contents below 40% (Akdogan, 1999). Bhattacharya (2012) identified three groups of extrusion cooked products based on their extrusion moisture conditions: low moisture extrusion cooking (up to 25% in-barrel moisture) of low density products, intermediate moisture extrusion cooking (between 25 and 40% in-barrel moisture contents) of expanded yet dense products and high moisture extrusion cooking (above 50% in-barrel moisture conditions) of soft and elastic products. Extrusion cooking at high moisture conditions will have a different effect on product physical, functional and nutritional properties than at low moisture, due to material in the barrel being less viscous which results in less pressure build-up (Akdogan, 1996). Barrel pressure is assumed to be directly proportional to the viscosity of the dough inside the barrel (Bhattacharya & Hanna, 1987).

Of necessity, other important parameters such as temperature are also adjusted between high and low moisture extrusion cooking in order to achieve the desired product from each unique processing technique. Table 2.6 shows how different products produced by twin screw extruders require different processing parameters such as temperature, in-barrel moisture contents, feed rate, screw speed and die geometry. As mentioned, these extruder variables have an influence on the cooking and therefore product nutritive, physical and functional quality (Mościcki & Van Zuilichem, 2011).

The composition of the raw materials that will form the viscous melt in the extrusion barrel is vital because this melt will form the basic structure of the end product (Guy, 2001). The melt comprises biopolymers broken down to more useful polymer sizes to form the desired structure. The melt can be considered a three-dimensional cage consisting of one main polymer, either starch or protein, which holds other materials (Guy, 2001). Biopolymers within a material interact with each other in the extrusion cooking process and should be considered as a system, rather than individually. Table 2.6 also shows how, not only process parameters, but also the composition of raw materials contribute to achieving different products in twin screw extrusion cooking. Guy (2001) grouped ingredients according to their functional role in forming the end product (Table 2.7).

Table 2.6 Independent extrusion cooking variables of four different products produced by twin screw extrusion cooking.

Product	**Expanded snack**	**Porridge flour**	**Pasta-like product**	**Meat analogue**
Raw materials (starch: protein ratio) (%)	66:12	64:17	69:12	10:90
Temperature from feeder to die (°C)	45, 85, 110, 140, 140	130 (die) and 165 (die)	30,90,90/100/110, 95	27, 60, 107, 129, 138/149/160
In-barrel moisture contents (%)	17	20	24-31	60-70
Feed rate (kg/h)	80	25.8	22.7-31.8	6.8
Screw speed (rpm)	400	200	100-150	150
Die opening (mm)	3.2	5	2.38 or 1.09	Cooling die
Drying	105 ˚C, 5 minutes	2 hours room temperature to cool	dried at ambient temperature until 10% moisture	40 °C for 24 hours until 10% moisture
Reference	Devi, Shobha, Tang, Shaur, Dogan and Alavi (2013)	Pelembe, Erasmus and Taylor (2002)	Wang, Bhirud, Sosulski and Tyler (1999)	Lin, Huff and Hsieh (2002)

Table 2.7 Guy classification system of raw materials used in extrusion cooking according to their functional role (Guy, 2001)

Group	Example	Functional role
1. Structure forming materials	Starch	Expandable biopolymer to allow bubbles to form and extend as it exits the die. A fall in temperature at this point causes a rise in viscosity, leading to a glassy state able to retain the expanded structure.
	Protein	Above 40% composition applied at 30-40% moisture. Globular proteins form continuous structures but are smaller than starch polymers. The proteins, therefore, link together and aggregate to form viscous complexes and films upon cooling to retain alveolar structured expansion.
2. Dispersed phase filling materials	Protein, starch, fibres	Lies within the continuous structure forming phase. Proteins tend to break into smaller molecules where fibre tends to remain firm and not always reduced in size. The presence of dispersed phase in cell walls could hinder expansion by disrupting cell walls or by altering the elasticity of the melt when exiting the die.
3. Plasticisers and lubricants	Water, fats and oils	Water above 25 % is regarded as a plasticiser which reduces the build-up of mechanical energy and in turnreduce heat generation. It also aids in dispersal of polymers. Oils and fats at 1-2% and higher are regarded as lubricants which cause 'slipping' to reduce applied shear.
4. Soluble solids	Salt and sugars	These substances are soluble in free water. At very high concentrations, these substances could replace water interactions with starch, altering the viscosity.
5. Nucleating substances	Calcium carbonate or magnesium silicate	These substances increase the quantity of bubbles formed in the expanding melt fluid. The compounds remain insoluble, providing a nucleation site where the energy required to form an individual bubble is reduced.
6. Colouring substances	Added or naturally present	Colouring substances are usually heat-stable colours added or already present in the raw material. These substances also include the precursors of colour formation by thermal reactions (Maillard).
7. Flavouring substances		Very similar to colouring substances. Flavouring substances are often added after the extrusion process.

2.4.2 Factors affecting physical and functional quality with a specific focus on sorghum-based extruded food products

Results obtained from particular extrusion cooking studies are dependent on the equipment, processing parameters and raw materials and are therefore difficult to compare (Falcone & Phillips, 1988). Some general trends are, however, applicable in many different extrusion cooking systems. Trends in functional, physical and later nutritional properties of sorghum-based extrusion systems will be reviewed, focusing on the impact of raw materials and in-barrel extrusion moisture contents on these properties. Where research in sorghum systems is lacking, trends in other extrusion systems will also be mentioned.

In many extrusion cooking applications including sorghum-based systems, starch is the structure forming material (Guy, 2001). The fate of the starch needs, therefore, to be understood. It has been presumed that the gelatinisation of starch (Pelembe et al., 2002) and the viscosity of the resulting starch melt in the extruder barrel (Mahasukhonthachat et al., 2010) have implications on the physical and functional properties of the resulting product. Extrudate expansion ratio measures the product's expansion in one dimension (axial). In a starch based-system, extrudate expansion is dependent on the formation of a starch matrix that traps water vapour, which results in the formation of bubbles (Guy, 2001). In a rice-sorghum-soy flour blend, expansion ratio was found to be related mainly to extrusion in-barrel moisture and temperature (Omwamba & Mahungu, 2014). In single-screw extrusion cooking of sorghum (Zamre et al., 2012), sorghum-cowpea blend (Falcone & Phillips, 1988) and sorghum-groundnut-cowpea blends (Asare et al., 2010), an increase in extrusion in-barrel moisture (12 to 16%, 20.5 to 25% and 12 to 44% moisture, respectively) caused reduced extrudate expansion. With increasing moisture conditions, the viscosity of the food melt decreases (Mahasukhonthachat et al., 2010), probably to a degree where it loses the ability to retain bubbles formed by moisture flash-off.

Extrudate density measures expansion in all directions as opposed to one dimension in expansion ratio (Falcone & Phillips, 1988). In research on sorghum-based extrudates, extrudate expansion ratio and density seem to be negatively correlated. Increased in-barrel extrusion moisture caused reduced expansion and increased density in single-screw extruded sorghum (Zamre et al., 2012). This trend was also found in sorghum-horsegram-defatted soy mix (9 to 21% moisture) (Basediya et al., 2011) and sorghum-maize mix (21 to 26% moisture) (Licata et al., 2014). Extrudate expansion ratio and density of extrudates also give insight into the macrostructure of extrudates. In maize-lentil extrudates, an increase in extrusion moisture caused thickening of extrudate air cell walls and a decrease in the number of air cells, resulting in larger air cells and a rigid structure (Lazou & Krokida, 2010b). It was proposed by the authors that the elasticity of the extrusion melt reduced with increased in-barrel moisture, causing cell walls to rupture and small air cells to merge, rendering a denser product.

Water absorption index (WAI) and water solubility index (WSI) give an indication of the changes in starch occurring in processing. Starch gelatinisation will increase water absorption, as hydrophilic groups are exposed; whereas starch degradation, which is the breakdown of starch polymers to smaller molecules beyond gelatinisation, yields soluble particles (Anderson et al., 1969). The fate of starch in extrusion cooking is thought to take place through a unique mechanism when compared to starch gelatinisation through hot pasting. The mechanism in extrusion relies on granule mechanical disruption due to high friction and shear, compared to the swelling of starch granules due to an excess of water in pasting (De Muelenaere, 1989). The extrusion mechanism is shown in Figure 2.4.

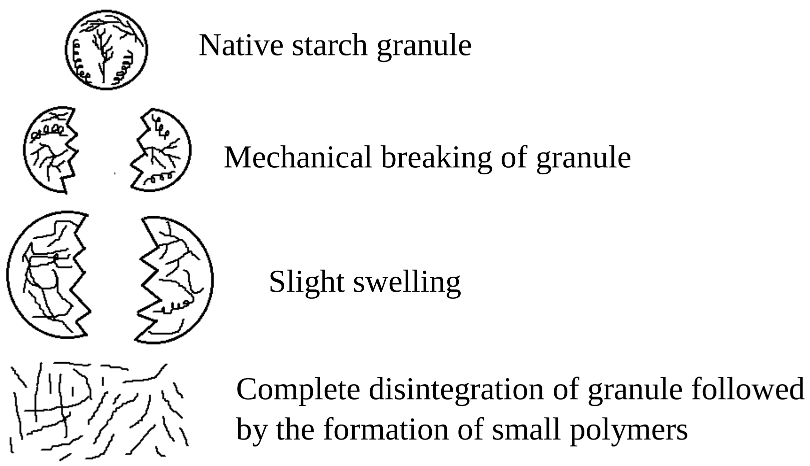


Figure 2.4 The fate of starch during extrusion cooking molecular fragmentation (De Muelenaere, 1989)

In research into a sorghum-soy system, it was proposed that increasing in-barrel moisture (14 to 20% moisture) caused a lower viscosity which enabled uniform mixing and heat distribution for better starch gelatinisation, therefore increasing WAI (Arun Kumar et al., 2015). In the same study, WSI was found to decrease with increasing feed moisture due to the lubricating effect of high moisture which lowered shear and inhibit starch degradation. However, it has also been proposed that increased in-barrel moisture (12 to 44% moisture) in sorghum-cowpea-groundnut composite extrusion reduced WAI, probably due to the lubricating effect of higher moisture in a single screw extruder (Asare et al., 2010). Mahasukhonthachat et al. (2010) also reported a decrease in WAI of sorghum extrudates as in-barrel moisture increased (25 to 40% moisture). In the same study, extrudate pasting properties showed a low initial peak viscosity (IV), but higher peak viscosity (PV), trough viscosity (TR) and final viscosity (FV) at higher moisture conditions.

The aforementioned functional and physical properties of extrudates are, however, not always solely dependent on the fate of starch in a system, but also on other macromolecules in the food matrix such as protein and the amount of these molecules that is present (Guy, 2001). An increase in protein in extrusion systems through the addition of legume was found to cause reduced extrudate expansion ratio in sorghum-cowpea (30 to 70% cowpea flour) (Pelembe et al., 2002), soy- or mixed legume-sorghum (15 to 30% legume flour) (Devi et al., 2013) and sorghum-soy (10 to 30 % soy flour) (Arun Kumar et al., 2015) systems. Extrudate density was found to correlate negatively with expansion ratio in sorghum-cowpea (33-67% cowpea flour) (Falcone & Phillips, 1988) and sorghum-soy (Arun Kumar et al., 2015) systems, where protein content increased through legume addition. Falcone and Phillips (1988) reported that the effect of increased in-barrel moisture (20.5-25% moisture) was greater than the effect of cowpea flour addition on properties such as extrudate density and expansion ratio. According to Omwamba and Mahungu (2014), the reduction in extrudate expansion and increase in density with protein addition is due to the dilution of starch. An increase in protein could also interfere with starch granule disruption (Hamaker & Bugusu, 2003), or, as suggested by Devi et al. (2013) could lead to protein-starch interactions, as also proposed by Allen et al. (2007). An increase in fat (Chinnaswamy & Hanna, 1988) and fibre (Mkandawire et al., 2015) has also been proposed to inhibit extrudate expansion. Pelembe et al. (2002) suggested that the addition of cowpeas to sorghum probably added more amylose to the food melt, also inhibiting expansion.

Even though the addition of legume flour causes increased extrudate density, Devi et al. (2013) suggested that the foaming capacity of proteins in legume flours could cause numerous air cells with a thin wall thickness in the macromolecular structure of sorghum-based extrudates.

The addition of legume flour also affects the performance of starchy extrudates in terms of WAI and WSI. WAI was found to increase with the addition of legume in sorghum-cowpea systems (Pelembe et al., 2002; Asare et al., 2010), wheat-sorghum and various legumes systems (Balasubramanian et al., 2011). Pelembe et al. (2002) suggested that the possible increase in amylose with the addition of cowpea caused increased WAI, whereas Asare et al. (2010)

suggested that it was due to the water binding effect of cowpea proteins. In contrast, an addition of soy of up to 30% to sorghum was found to cause reduced WAI, presumably due to the reduction of starch content (Arun Kumar et al., 2015). An increase in WSI with the addition of legume flours to sorghum (Pelembe et al., 2002) and sorghum-wheat mixtures (Balasubramanian et al., 2011) has been reported and it was proposed to be related to the solubility of legume flour proteins. Even though legume flour is expected to increase pasting curves due to its water binding capacity, sorghum-legume extrudates were found to give a lower overall viscosity of extrudate flours compared to sorghum alone (Balasubramanian et al., 2011). The authors suggested starch-protein interactions formed during extrusion cooking to be the reason for decreased overall viscosity.

The interactions between degraded starch and proteins also affect extrudate colour through non-enzymatic (Maillard and caramelisation) browning, depending on the severity of processing (Ames, 1998). The addition of legume flours to sorghum was found to only reduce extrudate redness caused by the sorghum (Pelembe et al., 2002). In contrast, an increase in moisture (12 to 16% moisture) was found, however, to cause darkening of sorghum extrudates (Zamre et al., 2012). Singh et al. (2007) summarised, however, that the effect of in-barrel moisture conditions on Maillard reaction is undefined and does not follow a definite trend.

Some functional assays applied to extrudates give an indication of the fate of protein specifically. Nitrogen solubility index (NSI) measures the solubility of proteins and indicates the extent of protein denaturation that has taken place due to food processing (Smith et al., 1966). The fate of protein in extrusion depends on the increased heat and shear in the extrusion barrel which weakens the tertiary and quaternary structures of proteins (Camire, 1991). Protein molecules subsequently unfold and align with the extrusion melt flow (Fig. 2.5).

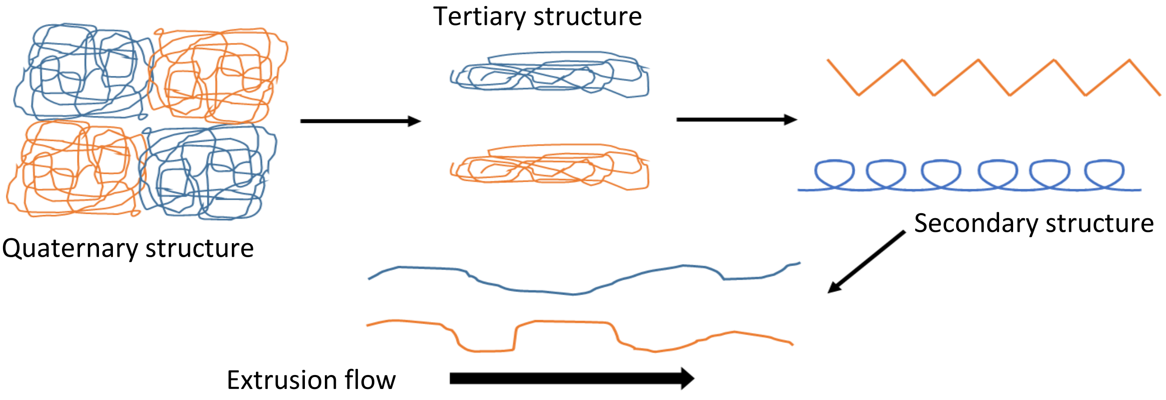


Figure 2.5 The fate of protein in extrusion cooking, based on Camire (1991)

NSI was found to increase in systems where legume flours were added to sorghum flour (Pelembe et al., 2002; Nicole et al., 2010), probably due to the soluble nature of legume proteins (Salunkhe & Kadam, 1989). Increasing in-barrel moisture has been found to cause higher NSI compared to low moisture conditions in extrusion cooking of cowpea, mung bean and defatted soy (30 to 45% moisture) (Pham & Del Rosario, 1984), navy, pinto and garbanzo beans (20 to 33% moisture) (Gujska & Khan, 1991) and peas (14 to 33% moisture) (Van der Poel et al., 1992). This was again probably due to the lubricating effect of moisture, which renders high moisture extrusion a less severe processing treatment with lower pressure and shear conditions (Guy, 2001).

2.4.3 Factors affecting nutritional quality with a specific focus on sorghum-based extruded food products

As in-barrel moisture affects the severity of extrusion as described above, it will affect extrudate nutritional quality by reduction in anti-nutritional factors, by changes in macromolecules which affect their digestibility and by chemical reactions which could alter nutrients.

Concerning phenolics, an increased in-barrel moisture in the extrusion cooking of whole grain red sorghum (14 to 19%) (Llopart et al., 2014) and peas, chickpeas, faba and kidney beans (14 to 20%) (Abd El-Hady & Habiba, 2003), was found to cause a large decrease in assayable phenolic compounds. In single screw extrusion cooking of sorghum at an in-barrel moisture of 15%, phenolic compounds were more extractable than at 18%, presumably due to their depolymerisation (Dlamini et al., 2007). However, extrusion cooking at 18% moisture probably promoted polymerisation of phenolics, rendering them less extractable. Llopart et al. (2014) proposed that greater interaction between protein and phenolics compounds occurs at lower moisture extrusion cooking conditions.

Concerning phytate, an observed reduction in phytate in red sorghum was found to be dependent on temperature increase rather than on in-barrel moisture content of extrusion cooking (Llopart et al., 2014). The effect of increased in-barrel moisture content in extrusion cooking of cereal brans (18 to 22% moisture) (Kaur et al., 2015) and various legumes (14 to 20% moisture) (Abd El-Hady & Habiba, 2003), was, however, found to cause a reduction in phytate. Decreases in phytate were found to be due to the hydrolysis of inositol hexaphosphates into lower phosphates (Alonso et al., 2001) or due to the formation of insoluble complexes between phytate and other charged components (Kaur et al., 2015).

With regard to protein quality, in-barrel moisture (15 and 25%) did not have a significant effect on *in vitro* protein digestibility (IVPD) (Fapojuwo et al., 1987; Dahlin & Lorenz, 1993). Extrusion cooking has, however, been suggested to disrupt the sorghum protein bodies due to high heat and shear (Hamaker et al., 1994). Disruption subsequently exposes the more soluble α-kafirin located in the centre of protein bodies, rendering the protein more accessible to digestive enzymes. The extent to which extrusion cooking favours or hinders protein digestibility is thought to be dependent on the severity of extrusion conditions (Taylor & Daiber, 1992). In the extrusion of pinto bean meal, an increase in extrusion moisture (18 to 22%) caused an increase in IVPD (Balandran-Quintana et al., 1998). The positive effect of increasing moisture is probably due to less severe conditions limiting amino acid reduction through reactions such as Maillard (Björck & Asp, 1983). Similarly, in a 70:30 sorghum malt-bambara groundnut composite, the loss of amino acids such as lysine and methionine was lower as in-barrel moisture increased (20-30% moisture) (Jiddere & Filli, 2015).

Concerning the effect of extrusion cooking on mineral bioavailability in sorghum extrudates, three studies found that extrusion cooking either had no effect on iron dialysability (Llopart et al., 2014) or caused an increase (Elobeid et al., 2014; Vilakati et al., 2016). Regarding the zinc dialysability of sorghum, extrusion cooking in a single screw extruder was found to cause a decrease (Llopart et al., 2014) and in a twin-screw extruder, an increase (Vilakati et al., 2016). The improvement of mineral bioaccessibility is possibly due to change in the structure of fibre polymers during extrusion cooking which alters their chelating properties, in turn decreasing fibre's inhibitory effect on mineral bioavailability (Alonso et al., 2001) or due to a reduction in phytates and phenolics.

Compositing cereals such as sorghum with legumes also has an effect on the nutritional quality of their extrudates. The addition of soy to a maize-sorghum blend was found to increase IVPD (Nicole et al., 2010). Increased protein digestibility is probably due to the more soluble nature of legume proteins (Salunkhe & Kadam, 1989). The addition of bambara groundnuts to sorghum malt was also found to increase extrudate lysine content (Jiddere & Filli, 2015). Also, lysine content and protein content was found to increase substantially upon compositing extruded sorghum flour with micronized cowpea flour (Vilakati et al., 2015). Compositing sorghum with legumes increases lysine because legumes are far richer in lysine compared to cereals (Salunkhe & Kadam, 1989). Although compositing with legumes will cause an increase in trypsin inhibitors, many studies have shown a large reduction in trypsin inhibitor activity after extrusion cooking (Alonso et al., 1998; Singh et al., 2007)

Concerning minerals, the addition of micronized cowpeas to extruded sorghum (Vilakati et al., 2016) and the addition of extruded white beans to extruded sorghum (Elobeid et al., 2014) were found to result in increased mineral contents. This is due to the higher mineral content of some legumes (Lestienne et al., 2005).

2.5 Conclusions

PEM and mineral deficiencies remain burdens in developing regions, especially in Africa. The low consumption of foods rich in high-quality proteins and minerals among children needs to be addressed to help prevent malnutrition. As a local African pulse, bambara groundnut, which have good protein quality and a relatively high iron content, is an important vehicle to address the nutritional shortcomings of cereals such as sorghum. Twin screw extrusion cooking is a high throughput, continuous food manufacturing process that could utilise bambara groundnuts and sorghum for the production of nutritious, convenience-type foods for the rapidly urbanising communities in Africa. Raw material composition and processing conditions in extrusion cooking, such as in-barrel moisture content, have major effects on the physical, functional and nutritional properties of extrudates. Little research has been done in starch-based systems on the effect of large moisture variations in extrusion cooking on extrudate properties, especially on the nutritional quality outcomes of extrusion systems with increasing moisture levels above 25%. The application of bambara groundnuts in such extrusion systems has not been studied. There is, therefore, a need to determine what the effect of large variations in feed moisture (15 to 20% moisture differences) and the inclusion of bambara groundnuts will have on extrudate physical, functional and nutritional properties. It is also necessary to evaluate whether the extrusion conditions that produce the best nutritional quality extrudates, also produce extrudates which can be applied as ready-to-eat foods in respect of their functional and physical properties.

3 HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

1. The inclusion of whole grain bambara groundnut flour with decorticated sorghum flour will improve the nutritional properties of the resulting extrudates in terms of protein and mineral contents and protein quality. Bambara groundnuts are richer in proteins and minerals (Falade & Nwajei, 2015) when compared to sorghum (Serna-Saldivar & Rooney, 1995). The more soluble globulin-type proteins of bambara groundnuts (Poulter, 1981) are probably more digestible after cooking and are richer in lysine (Yao et al., 2015) than the kafirin prolamin proteins of sorghum (Hamaker et al., 1986).

2. Extrusion cooking at low moisture will improve mineral bioaccessibility, but reduce *in vitro* protein digestibility compared to extrusion at high moisture. Extrusion cooking at low moisture is more severe due to more shear and heat build-up which cause chemical change and nutritional damage to the extrusion melt (Cheftel, 1986). The resulting large reduction in anti-nutritional factors will free more minerals for absorption. The improvement in protein digestibility by extrusion cooking of legumes (Abd El-Hady & Habiba, 2003) and sorghum (Hamaker et al., 1994) will be greater at high extrusion moisture, due to a smaller reduction in essential amino acids caused by Maillard reactions (Björck & Asp, 1983). Maillard reaction products can also inhibit proteolytic enzymes therefore reducing protein digestibility (Öste et al., 1986).

3. The inclusion of whole grain bambara groundnut flour with decorticated sorghum flour will decrease extrudate expansion and increase water absorption of extrudate flours. The expected increase in protein, fibre and lipids with the inclusion of whole grain bambara groundnuts (Falade & Nwajei, 2015) will dilute starch. Consequently, less starch will be available for gelatinisation to support an expanded extrudate structure (Guy, 2001). More hydrophilic structures present in legumes, such as protein, fibre and amylose, will lead to higher water absorption of extrudate flours as suggested by Asare et al. (2010).

4. Extrusion cooking at low moisture will result in greater extrudate expansion and extrudate flours will exhibit higher water absorption compared to extrusion at high moisture. The high pressure build-up in low moisture extrusion causes extensive starch gelatinisation in the barrel (Cheftel, 1986) and high extrudate expansion as extrusion melt leaves the die (Colonna et al., 1989). With high moisture extrusion, however, the low extrudate melt viscosity

(Mahasukhonthachat et al., 2010) will cause a reduced pressure drop at the die and decreased structure-forming abilities of the extrudate melt (Lazou & Krokida, 2010b) resulting in lower extrudate expansion.

3.2 Objectives

1. To determine the effects of compositing decorticated sorghum flour with whole grain bambara groundnut flour and of extrusion cooking at different in-barrel moisture conditions on the protein and mineral nutritional quality of extrudates, with the aim of addressing protein and mineral deficiencies in cereal-based diets of children aged 2-5 years.

2. To determine the effects of compositing decorticated sorghum flour with whole grain bambara groundnut flour and of extrusion cooking at different in-barrel moisture conditions on the physical and functional properties of extrudates to establish which conditions will produce extrudates which are suitable for convenience-type products.

4 RESEARCH

Effects of Bambara groundnut inclusion and different in-barrel extrusion moistures on nutritional, physical and functional properties of sorghum extrudates

ABSTRACT

Protein-energy malnutrition (PEM) and micronutrient deficiencies remain burdens among children in Africa due to cereal-based diets. Compositing with local pulses such as bambara groundnuts is a solution. Extrusion cooking can produce convenience-type products from local grains demanded by urbanised African communities. Decorticated sorghum and whole grain bambara groundnut flours were extruded separately and as a 50:50 composite using a twin screw extrusion cooker. The effect of flour composition and low (24%) and high (40%) in-barrel extrusion moisture on extrudate nutritional, physical and functional properties were studied. Compositing sorghum with bambara groundnuts increased protein by 54% and minerals by 118%. with zinc, calcium and magnesium increased by 32, 52, and 11%, respectively. *In vitro* protein digestibility and nitrogen solubility index increased substantially with bambara groundnut inclusion due to the soluble globulin-type proteins in bambara groundnuts. Phytate content was reduced by 12-35% in the composite and bambara groundnut extrudates after extrusion cooking at both extrusion moistures, possibly due to hydrolysis of inositol hexaphosphate. Caco-2-cell zinc bioaccessibility decreased with bambara groundnut inclusion, presumably due to increased Phy x Ca/Zn ratios. High extrusion moisture and bambara groundnut inclusion reduced extrudate expansion, probably due to lower viscosity in the extruder barrel and dilution of starch, respectively. High extrusion moisture yielded higher overall water absorption index, and higher peak, trough and final paste viscosities, probably due to reduced starch dextrinization. Bambara groundnut inclusion caused lower peak, trough and final paste viscosities, possibly due to starch dilution. Composite flours extruded at low in-barrel extrusion moisture produced extrudate flours with lower paste viscosity, suitable for nutrient-dense instant porridges and maintained high extrudate expansion for expanded snacks. Sorghum-bambara groundnut composite flour extruded at low in-barrel extrusion moisture produces protein-rich extrudates which could be applied as convenience-type products to address PEM.

4.1 INTRODUCTION

Sorghum is quantitatively the second most important cereal in Africa after maize when production statistics of 2014 are considered (FAOSTAT3, 2014). Although sorghum is an important source of nutrition in many African households, sorghum proteins are deficient in the indispensable amino acid, lysine (18 mg/ g sorghum protein) (Elkin et al., 1996) contributing to the cause of protein-energy malnutrition. Additionally, the lysine-rich germ and pericarp (Taylor & Schüssler, 1986) are removed in milling processes of sorghum which are usually applied to render sorghum more palatable (Taylor, 2003). Wet cooking, which is often applied in the preparation of traditional sorghum meals, causes a 16-41% decrease in sorghum protein digestibility (Chibber et al., 1978).

Legumes can supplement protein poor diets (Iqbal et al., 2006). The bambara groundnut is a drought tolerant, nutritious and underutilised indigenous African grain legume with high protein content (Jideani & Diedericks, 2014). Bambara groundnuts have a higher lysine content than sorghum (up to 1.5 g/100g whole grain) (Yao et al., 2015), with an *in vitro* protein digestibility of approximately 77% (Nwokolo, 1987), which probably increase upon cooking, as in most legumes (Liener, 1976).

The poor absorbability of iron and zinc in plant-based diets is, however, of great concern as it contributes to serious micronutrient deficiency (Hunt, 2003). Anti-nutritional factors such as polyphenolic compounds (Khokhar & Owusu Apenten, 2003) and phytates (Lopez et al., 2002) present in plant foods, form complexes with minerals, rendering them poorly available for absorption.

Extrusion cooking is a high-temperature-short-time technology which can produce shelf-stable convenience-type products (Kazemzadeh, 2012) from a variety of raw materials (Reimerdes, 1990). The shear, friction and heat which are generated in the extrusion barrel (Chessari & Sellahewa, 2001) can improve the digestibility of plant proteins (Abd El-Hady & Habiba, 2003), including sorghum proteins (Vilakati et al., 2015). A reduction in anti-nutritional factors such as tannins and phytates has also been achieved through extrusion cooking (Abd El-Hady & Habiba, 2003). Slight differences in raw material (Guy, 2001) or process variables (Chessari & Sellahewa, 2001) in the extrusion cooking process have a great impact on the nutritional, physical and functional properties of extrudates. Extrusion moisture is probably the process variable that has the greatest impact (Bhattacharya, 2012).

This study aims to determine the effects of bambara groundnut inclusion with sorghum and of extrusion cooking at low (24%) and high (40%) in-barrel moistures on protein and mineral contents and quality with respect to digestibility and *ex. vivo* Caco-2 cell uptake, respectively. The physical and functional properties of the resulting extrudates are also investigated to explore possible applications as convenience-type food products.

4.2 EXPERIMENTAL DESIGN

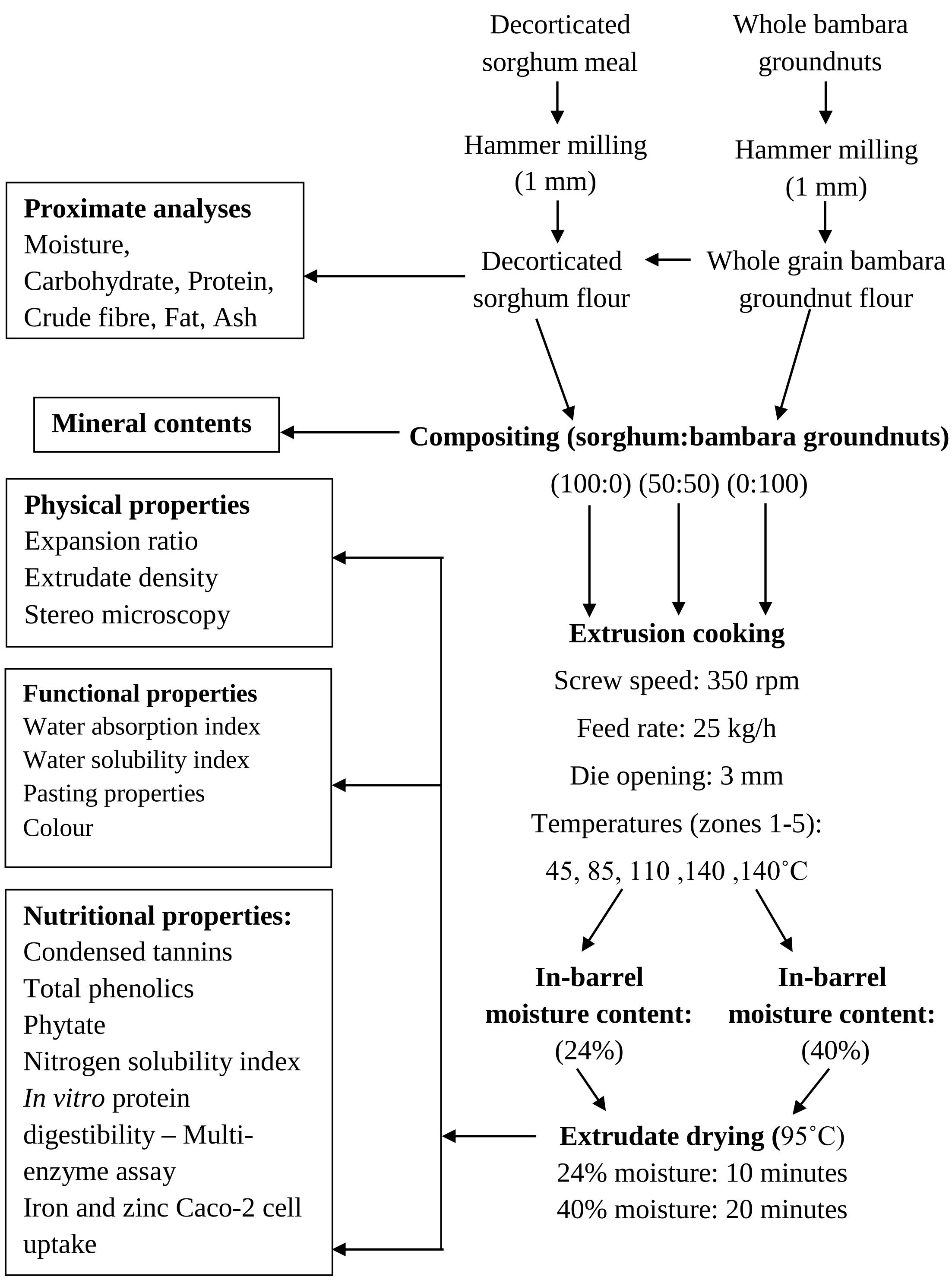


Figure 4.2.1 Flow diagram of experimental design from raw material preparation, compositing, extrusion cooking conditions and drying to analyses of extrudate physical, functional and nutritional properties

4.2.1 Materials

4.2.1.1 Grains

Decorticated (approximately 85% extraction rate) red non-tannin sorghum, 'Monati Super Fine Mabele Meal', was procured from Nola (Randfontein, South Africa). The meal was hammer milled using a Drotsky S1 Hammermill (Alberton, South Africa) fitted with a 1 mm mesh size screen to ensure a particle size of 1 mm or smaller, suitable for extrusion cooking through a 3 mm die.

Whole bambara groundnuts grown in Zimbabwe were procured from Triotrade (Silverton, South Africa). Stones and other debris were removed before the bambara groundnuts were hammer milled twice (1 mm mesh size screen). Prepared flours were stored at 4 °C.

4.2.1.2 Particle sizes of flours

The particle size distribution of the decorticated sorghum flour and whole grain bambara groundnut flour were determined according to the ASAE Standard S319 (ASAE, 1969). One hundred grams of the two flours were sieved on stacked 1 mm, 710 µm, 500 µm, 250 µm and 106 µm mesh size sieves for forty minutes using a mechanical shaker (Fritsch, Idar-Oberstein Germany). Three rubber band balls were used on each sieve to break up agglomerates of flour. The particle size distributions are recorded in Table 4.2.1. Both flours yielded almost no particles larger than 1 mm.

Table 4.2.1 Particle size distribution of sorghum and bambara groundnut flours

Particle size range (x in µm)	Fraction of flour (g/100 g)	
	Sorghum[1]	**Bambara groundnut[1]**
>1000	0.1 ± 0.1	1.5 ± 1.0
710 < x < 1000	8.0 ± 11.1	14.7 ± 13.9
500 < x ≤ 710	20.7 ± 7.3	19.6 ± 20.8
250 < x ≤ 500	57.0 ± 11.4	38.0 ± 13.9
106 < x ≤ 250	10.5 ± 3.3	22.5 ± 6.2
≤106	3.8 ± 5.8	3.9 ±2.7

1 Mean ± SD (n=3)

4.2.1.3 Compositing

Compositing decorticated sorghum flour with whole grain bambara groundnut flour was carried out based on weight in a 50:50 ratio. The flours were mixed for 10 minutes using an industrial mixer (Talsa, Mix 90 ST, Zaratamo, Spain) with a dual-armed paddle system – one arm stirs the product from the bottom to the top of the bowl, while the other arm simultaneously stirs product from the top to the bottom.

Homogeneity of the composite flour was tested through protein determinations of the individual flours and the composite. Protein was determined as explained in section 4.2.3.1. The protein content of the composite flour (17.0 g/100g) was similar to+ the calculated average protein content (17.0 g/100 g) of decorticated sorghum (11.3 g/100g) and whole grain bambara groundnut (22.8 g/100g) flours:

$$\frac{11.3+22.8}{2} = 17.1 \text{ g}/100\text{g}$$

4.2.2 Extrusion cooking of sorghum and bambara groundnut flours

A co-rotating twin screw extrusion cooker model TX 32 (CFAM Technologies, Potchefstroom, South Africa)(L/D = 21.5:1) was used which enabled extrusion cooking of coarse flours. The screw configuration is shown in Figure 4.2.2 and the extruder heats its barrels electrically. A twin screw extruder was used as they provide effective control of parameters to achieve the desired end product (Fellows, 2009).

Decorticated sorghum flour alone, a 50:50 composite of decorticated sorghum and whole grain bambara groundnut flours, and whole grain bambara groundnut flour alone were extruded separately. Extrusion cooking was carried out at two feed moistures for all three flours – low (24%) in-barrel moisture and high (40%) in-barrel moisture using an in line moisture pump. Preconditioning was not applied. Moisture was adjusted through calculating the amount of moisture feed necessary to achieve the aimed amount of in-barrel moisture in extrusion cooking and setting the correct moisture feed rate for each flour. The moisture feed was calculated using the equation from AACC International (2000) Method 26-95. Detailed extrusion cooking process conditions are given in Table 4.2.1. Due to limited time and raw materials only two extrusion runs were performed for each condition. Images of the extrusion cooking process are shown in Figure 4.2.3. The sample and moisture feeders were calibrated before extrusion cooking trials by measuring weight per minute.

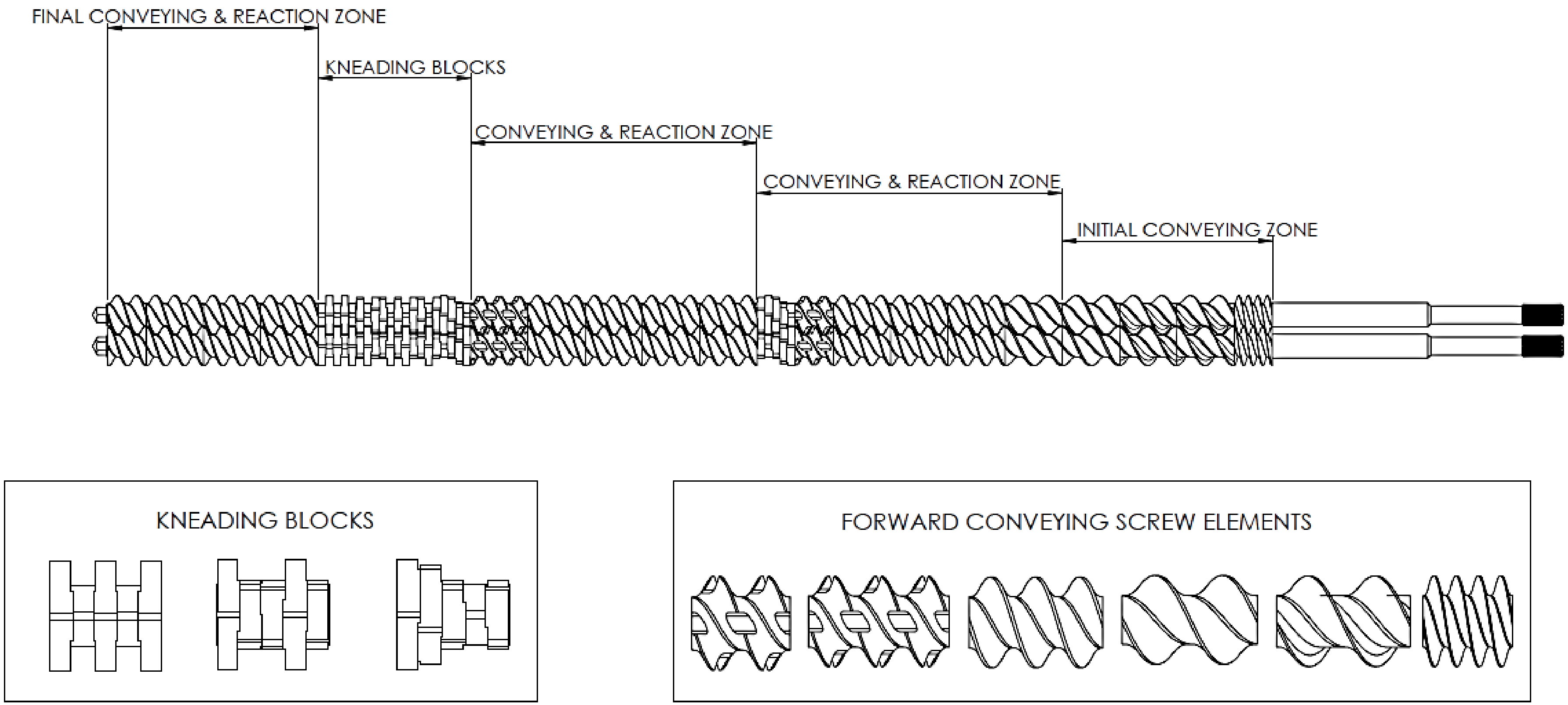


Figure 4.2.2 Screw configuration of screws used for extrusion cooking with a co-rotating twin screw extruder (CFAM Technologies, TX 32, Potchefstroom, South Africa)

Extrudates were dried at 95 ˚C in a convection oven set on humidity extraction (Unox, Cadoneghe, Italy). Extrudates which were extrusion cookedat 24% and 40% in-barrel moisture were dried for 10 and 20 minutes, respectively to achieve a final moisture content below 10%. Dried extrudates were ground in an air-cooled coffee-type grinder to a particle size of <710 µm for analyses.

Table 4.2.2 Extrusion cooking processing conditions for producing expanded decorticated sorghum, whole grain bambara groundnut and their composited extrudates

Processing conditions	**Parameter**
Feed rate	25 kg/h
Screw speed	450 rpm
Die hole diameter	3 mm
Barrel zone temperatures	
Zone 1	60 ˚C
Zone 2	80 ˚C
Zone 3	110 ˚C
Zone 4	140 ˚C
Zone 5	140 ˚C

Table 4.2.3 shows the important processing conditions during the two extrusion cooking runs. It seemed that the feed rates for 100% whole grain bambara groundnuts extruded at 24 % in-barrel moisture (replicate 1) and extruded at 40% in-barrel moisture (replicate 2) were somewhat lower and higher, respectively, compared to the other feed rates. The temperatures of zones 4 and 5 of 100% decorticated sorghum extruded at 40% in-barrel moisture were also somewhat lower than the set point temperatures.

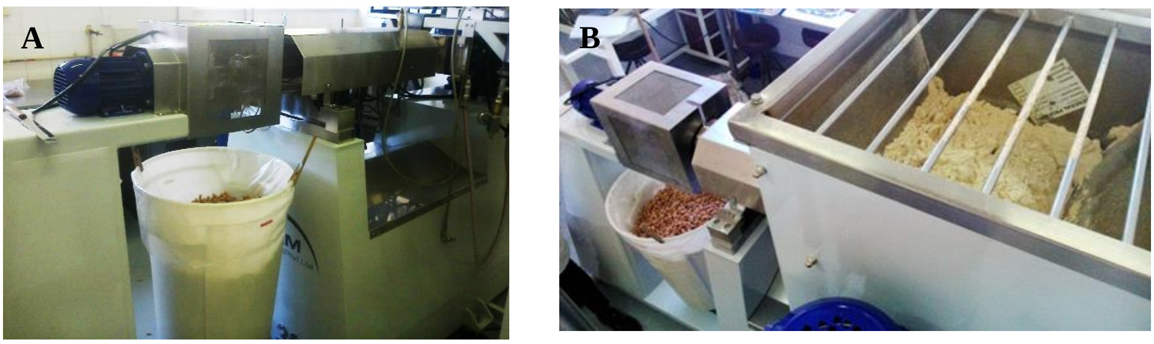


Figure 4.2.3 Extrusion cooker in process with the die end on the left of images

A: Side view, **B:** Top view

Table 4.2.3 Feed rates and zone temperatures during extrusion cooking processes of decorticated sorghum and whole grain bambara groundnut flours, respectively and composited at 24% and 40% in-barrel moisture

Flour composition	In-barrel moisture content (%)	R[1]	Feed rate (kg/h)	Temperatures of respective heating zones (˚C)				
				1 (SP[2]=60 ˚C)	2 (SP[2]=80 ˚C)	3 (SP[2]=110 ˚C)	4 (SP[2]= 140 ˚C)	5 (SP[2]=140 ˚C)
100% Decorticated sorghum	**24**	**1**	25.1 ± 1.3	56.4 ± 0.2	80.3 ± 0.3	110.4 ± 0.1	139.8 ± 0.3	140.8 ± 0.3
		2	24.9 ± 0.5	56.9 ± 0.5	81.8 ± 0.2	110.4 ± 0.5	139.5 ± 0.1	139.7 ± 0.2
	40	**1**	24.8 ± 0.5	56.2 ± 0.8	68.9 ± 1.1	103.4 ± 0.1	138.8 ± 0.2	137.7 ± 0.1
		2	24.6 ± 1.3	57.5 ± 0.7	71.4 ± 1.3	103.5 ± 1.1	136.1 ± 0.5	137.0 ± 0.3
50:50 Sorghum and bambara groundnut composite	**24**	**1**	24.6 ± 0.7	70.0 ± 3.4	87.1 ± 0.7	112.9 ± 0.4	137.6 ± 0.1	140.9 ± 0.3
		2	24.8 ± 0.9	57.9 ± 0.1	79.1 ± 1.1	109.1 ± 0.5	139.1 ± 0.6	138.0 ± 0.7
	40	**1**	25.0 ± 0.5	62.5 ± 0.2	76.7 ± 0.7	109.7 ± 0.1	141.8 ± 0.1	141.9 ± 0.1
		2	24.8 ± 0.6	55.4 ± 0.7	76.8 ± 2.5	110.6 ± 0.1	137.2 ± 0.3	139.9 ± 0.2
100% Whole grain bambara groundnuts	**24**	**1**	22.4 ± 0.8	53.9 ± 0.6	80.5 ± 0.3	111.7 ± 0.4	137.7 ± 0.4	141.5 ± 0.7
		2	24.3 ± 0.6	64.1 ± 0.8	86.9 ± 0.9	113.0 ± 0.4	142.7 ± 1.0	141.6 ± 0.2
	40	**1**	24.2 ± 1.1	60.6 ± 0.6	87.7 ± 0.1	111.6 ± 0.1	140.3 ± 0.3	140.3 ± 0.3
		2	26.7 ± 1.0	55.8 ± 0.5	71.8 ± 1.3	104.4 ± 2.5	139.0 ± 0.6	140.3 ± 0.4

[1] Replicate of extrusion cooking

[2] Set point temperature of the barrel zone during extrusion cooking

4.2.3 Chemical analyses

4.2.3.1 Proximate analyses

Moisture

The moisture contents of all raw and extruded flours were determined according to the AACC International (2000) air oven method 44-15A. Samples were accurately weighed close to 2 g and dried in a forced draught oven at 103 ˚C for 4 hours.

Ash

Ash contents of the decorticated sorghum flour and whole grain bambara groundnut flour were determined according to the AACC International (2000) Ash basic method 08-01. Approximately 2 g was accurately weighed into dried crucibles and charred on a tripod and gauze. Charred samples were heated at 550 ˚C for 5 hours until a light grey ash was obtained.

Protein

Protein contents of the raw and extruded flours were determined by a Dumas combustion method according to the AACC International (2000) Crude protein combustion method 46-30, using a factor of N x 6.25 for all samples.

Crude fat

The fat contents of whole grain bambara groundnut and decorticated sorghum flours were determined by a Soxhlet extraction method according to AOAC (2000) Official Method of Analysis 920.39.

Crude fibre

The crude fibre contents of whole grain bambara groundnut and decorticated sorghum flours were determined by a ceramic fibre filter method according to AOAC (2000) Official Method of Analysis 962.09.

Starch

The starch contents of decorticated sorghum and whole grain bambara groundnut flours were calculated as the difference between the total weight of each flour and the measured moisture, ash, fat, crude fibre and protein contents.

4.2.3.2 Tannin content

The 100% sorghum treatments were not analysed for total phenolic content or tannin content. This was because their amounts were expected to be insignificant due to the use of decorticated non-tannin sorghum flour (Awika et al., 2005a).

The modified vanillin-HCl assay of Price, Van Scoyoc and Butler (1978) was used to determine the tannin contents of all raw flours and of extruded composite and bambara groundnut flours. The assay depends on the reaction of vanillin with condensed tannins to form coloured complexes. The complexes are in turn quantified through spectrophotometry against a catechin standard. Extractions were carried out for 20 minutes at 150 rpm on a mechanical shaking tray. Reagent blanks for all samples were included to correct for the colour of the extracts. The samples were read at 500 nm. Tannin contents were expressed as g catechin equivalents (CE) per 100 g sample. Duplicate extracts of each treatment from each extrusion run were analysed.

4.2.3.3 Total phenolic content

For the determination of the total phenolic content, the same extracts were used as in the modified vanillin assay. The modified method of the Folin-Ciocalteu assay described by Singleton and Rossi (1965) was used. The assay relies on the reaction of the Folin-Ciocalteu reagent with phenols to form chromogens (Waterman & Mole, 1994). These were quantified spectrophotometrically, also against a catechin standard. The samples were read at 760 nm. Total phenolic contents were expressed as g CE equivalents per 100 g sample. Duplicate extracts of each treatment from each extrusion run were analysed.

4.2.3.4 Phytate content

The method of Frühbeck, Alonso, Marzo and Santidrián (1995) was modified. Extraction was carried out for 2 hours at 150 rpm on a mechanical shaking tray. Centrifugation was carried out at a speed of 14020 g and a temperature of 5 ˚C to separate fat from the supernatant. Anion-exchange purification was carried out using 0.3 g Dowex 1, anion-exchange resin-AG 1 x 4, 4 % cross-linkage, chloride form, 100-200 mesh (Sigma-Aldrich, Johannesburg, South Africa) in glass barrel Econo-columns, 0.7 x 15 cm (BioRad, Johannesburg, South Africa). A 1:10 dilution of the supernatants at pH 6 was passed through the anion-exchange column. To elute inorganic phosphate and phytate, 7.5 mL 0.1 N NaCl and 0.7 M NaCl were used, respectively. Phosphates from the isolated inorganic phosphates react with ferric chloride and sulphosalicylic acid, and chromogens are formed for spectrophotometric quantification.

Samples were read at 500 nm. Phytate content was expressed as g phytate per 100 g sample. Duplicate extracts of each treatment from each extrusion run were analysed.

4.2.3.5 In vitro protein digestibility

In vitro protein digestibility (IVPD) was determined by a multi-enzyme assay as described by Hsu, Vavak, Satterlee and Miller (1977). The enzyme solution used for digestion consisted of 1.6 mg/mL trypsin, 13 000-20 000 BAEE units/mg protein (T03030, Sigma-Aldrich), 3.1 mg/mL bovine chymotrypsin type II, 60 units/mg protein (C 4129, Sigma-Aldrich) and 1.3 mg/mL protease XIV, 3.5 units/mg solid (P5747, Sigma-Aldrich). The drop in pH due to the release of the amino acids from digested proteins in a non-buffered solution was quantified using a pH meter. IVPD was expressed as a percentage of the total amount of protein in each sample. Duplicates of each treatment from each extrusion run were analysed.

4.2.3.6 Nitrogen solubility index

Nitrogen solubility index (NSI) of extruded and raw flours was determined according to the AACC International (2000) NSI method 46-23. Flours were accurately weighed close to 1 g into 50 mL centrifuge tubes. Twenty mL 0.1 M NaCl was added to each tube and pH adjusted to 7 using 0.1 N HCl or NaOH. Tubes were shaken in a shaking water bath for 2 hours at 30 ˚C, centrifuged (10000 g, 15 min, 4 ^{o}C) and the supernatant filtered through a Whatman No. 4 filter paper. The pellets were subsequently washed with 10 ml 0.1 M NaCl with the pH maintained at 7, centrifuged, filtered and repeated. The combined filtrate of each sample was freeze dried to a dry powder. The powders were analysed for nitrogen content by combustion analysis using a Dumatherm instrument (Gerhardt, Konigswinter, Germany). Nitrogen solubility index was expressed as g soluble protein per g protein content of 100 g sample (db) (protein conversion factor N x 6.25). Duplicates of each treatment from each extrusion run were analysed.

4.2.3.7 Mineral contents

Nitric-perchloric acid digestion was performed according to Zasoki and Barau (1977) on sorghum, bambara groundnut and composite raw flours. Digests were diluted to 35 mL and filtered (Whatman, ashless, grade 40). The iron, zinc, calcium, phosphorus, magnesium, copper, manganese, aluminium and chromium contents of the samples were measured, using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Kruger et al., 2013).

Blanks were also included to correct for any minerals that could have been contributed through digestion or filtration. Each flour was analysed in triplicate.

4.2.3.8 Mineral uptake analysis

Cell culture

Caco-2 cells were cultured as described by Kruger et al. (2013). Caco-2 cells were kindly donated by the Department of Pharmacology, North West University (Potchefstroom, South Africa) and were cultured in Dulbecco's Modified Eagle Medium (DMEM) with glucose, Earle's salts and L-glutamine (Hyclone, Separations) containing 10% foetal bovine serum, which was not heat-treated (Highveld Biological, Pretoria, South Africa) and 1% antibiotics (Hyclone, Separations). Confluent cells were washed with phosphate-buffered saline (pH 7.2) and rinsed off the bottom of the flask with 2% trypsin. Cells for the assay were seeded in 24-well culture plates.

***In vitro* digestion of flours**

Raw and extruded flours were subjected to *in vitro* digestion as described by Kruger et al. (2013) to simulate human digestion. Flour solutions were made up with distilled water at 0.05 g/mL and dispersed using a mechanical shaking tray POS-300 (Grant Instruments, England) for 10 minutes at 150 rpm. The pH of samples was adjusted to 2.0 with 1N HCl. Enzymes used were 2% crystallised and lyophilised, essentially salt-free pepsin (porcine, 4200 U/mg, Sigma-Aldrich,) at 5 µL pepsin/mL sample and 0.4% pancreatin (P-1750, Sigma-Aldrich) at 5 µL pancreatin/mL sample.

Extrinsic labelling of flour digests

Digests were mixed in equal parts with the same medium used in cell cultures, except only 2% bovine serum was added instead of 10%. Labelling with ^{59}Fe and ^{65}Zn (Separations Scientific, Johannesburg, South Africa) were further followed as described by Kruger et al. (2013). In this study, 50 000 counts per minute (cpm)/mL were achieved.

Caco-2 cell mineral uptake and radioactivity measurements

Wells were incubated with labelled samples as described by Kruger et al. (2013). A Wizard 2470 automatic gamma counter (Wallac, Perkin Elmer, Johannesburg, South Africa) was used to count cells and samples:medium mixtures, respectively. At least 4 wells per sample were used for analysis. Positive and negative controls were also analysed to ensure that readings were in range. The positive control consisted of 25 μM $^{59}FeSO_4$ or $^{65}ZnCl_2$ and the negative control with additional 1mM phytate. Mineral uptake by Caco-2 cells, which indicated mineral bioaccessibility, was presented as the percentage of radioactivity in the cells of the total activity in the cells and sample medium after incubation.

4.2.3.9 Stereomicroscopy

Extruded samples which had not been milled were used for stereomicroscopy. Samples were cut longitudinally and cross-sectionally with a scalpel. Images of the cut samples and also of the surfaces of each extrudate was obtained at x 11.3 magnification using a Discovery V20 stereomicroscope (Zeiss, Götingen, Germany)

4.2.3.10 Extrudate expansion ratio

Extrudate expansion ratio (ER) for each treatment was determined by dividing the diameter of the extrudate by the diameter of the die (Colonna et al., 1989). Eighteen extrudates for each treatment were measured

4.2.3.11 Extrudate density

The extrudate density (ED) for every treatment was determined by measuring the mass (m), length (l) and diameter (d) of 18 extrudates per treatment. The following equation was used to determine piece density (Karkle et al., 2012):

$$\mathrm{ED}\ (\mathrm{kg/m^3}) = \frac{4\mathrm{m}}{\pi \mathrm{d}^2 \mathrm{l}}$$

4.2.3.12 Water absorbance index

Water absorbance index (WAI) was determined according to the method of Anderson et al. (1969) . Raw and extruded flours (2.5 g) were suspended in 30 mL distilled water at 30 ˚C in 50 mL centrifuge tubes. The tubes were shaken for 30 min in a shaking water bath maintained at 30 ˚C and agitated with a vortex 6 times in between. The tubes were centrifuged at 3147 g for 15 minutes and left overnight at ambient temperature for pellets and supernatants to separate completely. Supernatants were removed from the pellet using a Mohr pipette. Triplicates of each treatment from each extrusion run were analysed. The following equation was applied to determine WAI which was expressed in db:

$$\text{WAI}=\frac{\text{pellet weight (g)} - \text{sample weight (g)}}{\text{sample weight (g)}}$$

4.2.3.13 Water solubility index

Water solubility index (WSI) was also performed and calculated according to Anderson et al. (1969). The supernatant from the WAI centrifuged tubes was decanted into dry moisture tins. The supernatants were oven dried overnight at 100 ˚C and weighed. Triplicates of each treatment from each extrusion run were analysed. The weight of dried supernatant was used to express soluble material in grams per 100 g sample (db) through the following equation:

$$WSI = \frac{dried\ supernatant\ weight\ (g)}{sample\ weight\ (g)} \times 100$$

4.2.3.14 Pasting properties

The pasting properties of all raw and extruded flours were measured using a Rapid Visco Analyser (RVA model 3D+, Newport Scientific, Warriewood, Australia). Flour and distilled water suspensions were prepared at 12% solids to a total of 28 g. During the pasting procedure, suspensions were equilibrated at 50 ˚C for 1 minute, after which heating to 90 ˚C at a uniform rate of 5°C per min with constant stirring at 160 rpm, was applied. The heated slurry was held at 91°C for 7 minutes, then cooled to 50°C at 5°C per min. It was then held at 50°C for 2 minutes. Viscosity was recorded in cPa. Duplicates of each treatment from each extrusion run were analysed.

4.2.3.15 Colour

Colour of raw and extruded pasted flours were recorded using a Chroma meter CR-400 (Konica Minolta Sensing, Osaka, Japan). The L (lightness), a (redness-greenness) and b (yellowness-blueness) colour components were measured. Pasted slurries were decanted into Petri dishes. The chroma meter was calibrated using a white tile before measurements were taken. Measurements were randomly taken at the surface of the petri dish along the circumference. Three readings were taken for each petri dish.

4.2.4 Statistical analyses

Multivariate analysis using the factorial design (3 x 2 factorial) was used to analyse the interaction between the effect of different flours and in-barrel moisture contents on the physical, functional and nutritional characteristics. Two way analysis of variance (ANOVA) was used to determine the overall effect of flour composition and in-barrel moisture contents, respectively. Means were compared at $p \leq 0.05$ using Fisher's Least Significant Difference (LSD) test. Statistica Software version 10.0 (StatSoft, Tulsa, Arizona, USA) was used for Multivariate Analysis, Fisher's LSD test and Correlation.

4.3 RESULTS AND DISCUSSION

4.3.1 Nutritional properties

4.3.1.1 Proximate composition

Compared to decorticated sorghum, whole grain bambara groundnuts had significantly higher ($p \leq 0.05$) ash (mineral) (218% higher), protein (107% higher), fat (52% higher) and crude fibre (327% higher) contents (Table 4.3.1). Decorticated sorghum had a higher starch content (27% higher). The differences in composition between sorghum and bambara groundnuts were expected in light of previous research on sorghum (Serna-Saldivar & Rooney, 1995) and bambara groundnuts (Falade & Nwajei, 2015). The crude fibre content of the bambara groundnuts was, however, higher than that found by Falade and Nwajei (2015) but agreed with findings by Enwere and Hung (1996). Crude fibre, ash and fat contents of sorghum were also slightly lower compared to previous research (Adeyeye & Ajewole, 1992), probably due to the removal of bran and germ where these compounds are concentrated during decortication and milling (Serna-Saldivar & Rooney, 1995).

Table 4.3.1 Proximate composition of raw decorticated sorghum flour and whole grain bambara groundnut flour (g/100 g)

Flour[1]	Moisture	Ash	Protein	Crude fat	Crude fibre	Starch[3]
100% Decorticated sorghum	$12.4^{b2} \pm 0.2$	$1.1^{a} \pm 0.0$	$9.9^{a} \pm 0.4$	$2.7^{a} \pm 0.2$	$1.1^{a} \pm 0.1$	72.8
100% Whole grain bambara groundnuts	$10.0^{a} \pm 0.2$	$3.5^{b} \pm 0.0$	$20.5^{b} \pm 0.3$	$4.1^{b} \pm 0.4$	$4.7^{b} \pm 0.1$	57.2

[1] Mean ± SD (n=4, duplicate samples of 2 extrusion runs)
[2]Mean values with different lowercase superscript letters in a column differ significantly ($p \leq 0.05$)
[3]Calculated by difference of all other proximate components

4.3.1.2 Condensed tannins and total phenolics

Overall, extrusion cooking substantially reduced the assayable total phenolic (TP) contents by at least 85% (Table 4.3.2). Various authors have reported reductions in TP content in legumes and cereals and their composites such as navy and red bean flour and corn starch mixes (13-74% reduction) (Anton et al., 2009), faba and kidney beans (29-46% reduction) (Alonso et al., 2000) and whole grain non-tannin and tannin sorghum (up to 62% reduction in decorticated sorghum) (Dlamini et al., 2007) after extrusion cooking. A reduction in assayable TP does not necessarily point to the degradation of these phenolic compounds. Assayable TP may be decreased due to binding of phenolic components to macromolecules such as proteins or degradation, depolymerisation and polymerisation of phenolic components (Taylor & Duodu, 2015). In contrast to degradation where phenolic components are actually inactivated, complexing, depolymerisation and polymerisation can cause a reduction in extractability of phenolic components (Duodu, 2014). Depolymerisation can also cause phenolic components to be more extractable, leading to higher assayable TP content (Dlamini et al., 2007).

Overall, 40% in-barrel extrusion moisture caused a greater decrease ($p \leq 0.05$) in assayable TP content compared to low moisture extrusion cooking (Tale 4.3.2). This effect of extrusion moisture agreed with previous findings with various legumes (Abd El-Hady & Habiba, 2003) and in whole grain red sorghum (Llopart et al., 2014). With extrusion cooked sorghum, Dlamini et al. (2007) proposed that extrusion cooking at low moisture conditions (15%) caused depolymerisation of phenolics, whereas higher moisture (18%) promoted polymerisation of phenolics. It was further suggested that the depolymerised phenolics were more extractable than polymerised phenolics, possibly leading to increased assayable TP contents at higher extrusion moisture.

The effect of extrusion moisture was, however, only significant ($p \leq 0.05$) for the 100% bambara groundnut extrudates (Table 4.3.2). Bambara groundnuts extruded at 24% in-barrel moisture showed a smaller reduction in TP compared to the raw flour (77%) in comparison to the reduction in composite and sorghum flours (96-100%). Maillard products (Kim & Lee, 2009) in extruded bambara groundnuts, possibly caused a higher TP apparent by reacting with the Folin-Ciocalteu reagent (Waterman & Mole, 1994). A presence of more Maillard products in bambara groundnut extrudates was possibly caused by their higher protein contents (Table 4.3.1). At low extrusion moisture, Maillard reaction substrates are concentrated in the extruder barrel, yielding more Maillard products (Björck & Asp, 1983).

Table 4.3.2 Effects of extrusion cooking in-barrel moisture and of compositing on the anti-nutritional factors of decorticated sorghum flour and whole grain bambara groundnut flour

Characteristic	**Extrusion cooking treatment**	**100% Decorticated sorghum**	**50:50 Sorghum and bambara groundnut composite**	**100% Whole grain bambara groundnuts**	**Effect of in-barrel moisture content[3]**
Total phenolic content (g CE/100 g, db)[1]	Not extruded	0.19 ± 0.01	0.53 ± 0.03	0.81 ± 0.20	0.67 ± 0.20
	24 % in-barrel moisture	Not determined	0.02^{a2} ± 0.02	0.19^{b} ± 0.06	0.10^{B} ± 0.10
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	Not determined	None detected[a]	0.01^{a} ± 0.01	0.01^{A} ± 0.01
Effect of flour composition[4]		Not determined	0.01^{A} ± 0.02	0.10^{B} ± 0.10	
Condensed tannin content (g CE/100 g, db)[1]	Not extruded	None detected	None detected	0.12 ± 0.03	0.06 ± 0.07
	24 % in-barrel moisture	Not determined	0.14 ± 0.08	0.16 ± 0.07	0.15^{B} ± 0.07
Flour*moisture interaction p = 0.8768	40 % in-barrel moisture	Not determined	0.07 ± 0.08	0.10 ± 0.06	0.08^{A} ± 0.06
Effect of flour composition[4]		Not determined	0.11^{X} ± 0.08	0.13^{X} ± 0.07	

[1] Mean ± SD (n=4, duplicate samples from 2 extrusion runs)

[2] Mean values with different lowercase superscript letters in a block differ significantly (p≤0.05) (Flour*moisture interaction p-values stated under descriptor)

[3] Mean values with different uppercase superscript letters in a column differ significantly (p≤0.05)

[4] Mean values with different uppercase superscript letters in a row differ significantly (p≤0.05)

Further, the trend in the raw flours where TP increased ($p \leq 0.05$) with the inclusion of bambara groundnuts was retained with extrusion cooking (Table 4.3.2).

Condensed tannins showed high variability due to their very low levels (Table 4.3.2). The absorbance values obtained in this study were much lower than absorbance values which give repeatable results in the modified vanillin-HCl assay for Price et al. (1978). The condensed tannin content in the raw and extruded flours were probably of a negligible amount.

4.3.1.3 Phytate

Overall, extrusion cooking reduced phytate content by 7-14% (Table 4.3.3). The reduction by extrusion cooking was in accordance with the reduction of phytate contents observed with extrusion cooking of cereal brans (42 to 50% reduction) (Kaur et al., 2015) and pea and kidney bean seeds (4 to 21% reduction) (Alonso et al., 1998; Alonso et al., 2001). The reduction in phytate content by extrusion cooking has been shown, using high performance liquid chromatography, to be as a result of the formation of penta- and tetraphosphate through hydrolysis of inositol hexaphosphates (Sandberg et al., 1987; Alonso et al., 2001).

Overall, 24% extrusion moisture reduced extruded phytate content to a greater extent ($p \leq 0.05$) compared to 40% extrusion moisture (Table 4.3.3). This effect of extrusion moisture, however, contradicts previous research (Abd El-Hady & Habiba, 2003; Kaur et al., 2015) where higher extrusion moisture caused a greater reduction in phytate content. None of these investigations, however, studied an extrusion moistures as in the current study, with moistures being much lower (increases of 14 to 20% extrusion moisture and 18 to 22% extrusion moisture, respectively). The application of severe or prolonged heat is believed to play the main role in the hydrolysis of inositol hexaphosphate during food processing (De Boland et al., 1975). In this present work, it is possible that the lubricating effect at higher moisture reduced heat generated by friction (Fellows, 2009), causing a smaller decrease in phytate content.

Table 4.3.3 Effects of extrusion cooking in-barrel moisture and of compositing on the phytate content of decorticated sorghum flour and whole grain bambara groundnut flour

Characteristic	**Extrusion cooking treatment**	**100% Decorticated sorghum**	**50:50 Sorghum and bambara groundnut composite**	**100% Whole grain bambara groundnuts**	**Effect of in-barrel moisture contents[3]**
Phytate content (g/ 100 g, db)[1]	Not extruded	1.23 ± 0.10	1.46 ± 0.08	1.55 ± 0.12	1.45 ± 0.15
	24 % in-barrel moisture	1.36^{b2} ± 0.12	1.24^{b} ± 0.08	1.00^{a} ± 0.09	1.25^{A} ± 0.19
Flour*moisture interaction p = 0.00004	40 % in-barrel moisture	1.40^{b} ± 0.05	1.28^{b} ± 0.07	1.36^{b} ± 0.25	1.35^{B} ± 0.16
Effect of flour composition[4]		1.26^{Y} ± 0.07	1.20^{Y} ± 0.08	1.07^{X} ± 0.24	

[1] Mean ± SD (n=4, duplicate samples from 2 extrusion runs)

[2] Mean values with different lowercase superscript letters in a block differ significantly (p≤0.05) (Flour*moisture interaction p-values stated under descriptor)

[3] Mean values with different uppercase superscript letters in a column differ significantly (p≤0.05)

[4] Mean values with different uppercase superscript letters in a row differ significantly (p≤0.05)

Despite higher phytate content in the raw flour of bambara groundnuts (1.55 g/100 g) compared to sorghum (1.23 g/100 g), bambara groundnut extrudates had a lower phytate content overall ($p \leq 0.05$) compared to sorghum and composite extrudates (Table 4.3.8). The greater reduction in phytate content of bambara groundnuts after extrusion cooking could be due to the physiological difference in phytate of bambara groundnuts compared to sorghum. Phytates are stored in globoid crystals, which for legumes, are located in the cotyledons (Urbano et al., 2000), compared to cereals where globoid crystals are located in the aleurone layer and germ (O'Dell et al., 1972). Partly degermed sorghum was used in this study. Hídvégi and Lásztity (2003) also summarised that the formation of globoid crystals depends on different environmental factors.

4.3.1.4 Protein contents and quality

Bambara groundnuts had higher protein contents than sorghum which resulted in a substantial ($p \leq 0.05$) overall increase in protein (46%) content when compared with sorghum (Table 4.3.4). As would be expected, intermediate protein contents have been achieved in research where sorghum was composited with cowpeas (14-16% protein) (Pelembe et al., 2002; Vilakati et al., 2015), soy and lentil flour (22% and 12% protein, respectively) (Devi et al., 2013), and sugar beans (13% protein) (Jackson et al., 2013). As expected, the different in-barrel moisture contents did not cause a significant difference ($p > 0.05$) in protein contents, as extrusion cooking is a closed system where no nitrogen can be added or lost. The WHO/FAO/UNU Expert Consultation (2007) reported the average protein requirement for children aged 2 years and between 3-5 years to be 0.79 and 0.70 g protein/kg body weight per day, respectively. If the average weight for children between 2-5 years of age is 13-19 kg (WHO/UNICEF/UNU, 2001), the average protein requirement per day will translate to 10.3-13.3 g protein per day. A serving size of 50 g dry base composite extrudate could contribute approximately 81% of this protein requirement.

Table 4.3.4 Effects of extrusion cooking in-barrel moisture and of compositing on the protein content and *in vitro* protein digestibility (IVPD) of decorticated sorghum flour and whole grain bambara groundnut flour

Characteristic	**Extrusion cooking treatment**	**100% Decorticated sorghum**	**50:50 Sorghum and bambara groundnut composite**	**100% Whole grain bambara groundnuts**	**Effect of in-barrel moisture contents[3]**
Protein content (g/ 100 g, db)[1]	Not extruded	11.3 ± 0.4	17.0 ± 0.1	22.9 ± 0.3	17.0 ± 5.0
	24 % in-barrel moisture	13.4 ± 0.2	19.2 ± 1.1	21.9 ± 0.3	18.2^{A} ± 3.9
Flour*moisture interaction p = 0.3163	40 % in-barrel moisture	12.8 ± 0.2	19.0 ± 1.3	24.0 ± 2.5	18.6^{A} ± 5.2
Effect of flour composition[4]		13.1^{X} ± 0.4	19.1^{Y} ± 1.0	23.0^{Z} ± 1.9	
IVPD (%) Multi-enzyme assay[1]	Not extruded	81.5 ± 0.3	82.0 ± 0.3	81.8 ± 1.7	81.7 ± 0.9
	24 % in-barrel moisture	84.0^{a} ± 1.0	85.9^{b} ± 0.3	87.1^{c} ± 0.2	85.7^{A} ± 1.5
Flour*moisture interaction p = 0.0053	40 % in-barrel moisture	83.7^{a} ± 0.4	87.4^{c} ± 0.1	87.7^{c} ± 0.2	86.3^{B} ± 1.9
Effect of flour composition[4]		83.8^{X} ± 0.7	86.7^{Y} ± 0.8	87.4^{Z} ± 0.4	

[1] Mean ± SD (n=4, duplicate samples from two extrusion runs)

[2] Mean values with different lowercase superscript letters in a block differ significantly ($p \le 0.05$) (Flour*moisture interaction p-values stated under descriptor)

[3] Mean values with different uppercase superscript letters in a column differ significantly ($p \le 0.05$)

[4] Mean values with different uppercase superscript letters in a row differ significantly ($p \le 0.05$)

Extrusion cooking caused an overall increase (5-6%) in IVPD (Table 4.3.4) and a dramatic overall decrease (88%) in NSI (Table 4.3.5). Hot, moist conditions in extrusion cooking probably caused exposure of hydrophobic protein cores (Camire, 1991) and polymerisation after unfolding of the protein matrix (Stanley, 1989), which are believed to reduce protein solubility. Protein denaturation also possibly exposed more sites for proteolytic attack, improving IVPD (Hsu et al., 1977). The reduction of phytates (Oatway et al., 2001) as seen in Table 4.3.3 and the probable decrease of trypsin inhibitors after extrusion cooking (Batista et al., 2010b) possibly reduced the adverse effects of these anti-nutrients on protein digestibility. IVPD values for extruded composite flours were lower when compared to a 70:30 extruded sorghum-micronized cowpea composite (Vilakati et al., 2015), however, they were higher than 70:30 sorghum-cowpea flours which have been cooked into unfermented and fermented porridges (Anyango et al., 2011). As stated, the heat and shear of extrusion cooking are thought to disrupt the protein bodies of sorghum exposing more the α-kafirins to proteolytic attack (Hamaker et al., 1994). The α-kafirins are thought to be more digestible than the β- and γ-kafarins and is possibly related to the higher digestibility of α-kafirins, especially after extrusion cooking (Hamaker et al., 1994).

The effect of extrusion moisture on NSI was significant ($p \leq 0.05$) in bambara groundnut extruded flours where 24% in-barrel extrusion moisture yielded less soluble proteins ($p \leq 0.05$) than extrusion cooking at 40% extrusion moisture in (Table 4.3.5). This agreed with previous studies on beans extruded at different moisture contents (Pham & Del Rosario, 1984). This trend was only found with bambara groundnuts, probably due to its more soluble globulin type proteins (Yagoub & Abdalla, 2007). The higher extrusion moisture condition caused a significant overall increase ($p \leq 0.05$) in IVPD compared to low extrusion moisture (Table 4.3.4). This finding is similar to a study on whole pinto bean meals where IVPD increased with in-barrel extrusion moisture (18-22% moisture) (Balandran-Quintana et al., 1998). As stated, low extrusion moisture is believed to produce more severe cooking conditions due to greater heat generation as a result of more friction (Pham & Del Rosario, 1984). More severe conditions probably cause more protein denaturation, therefore lower solubility (Smith et al., 1966). Reduced friction and a dilution of Maillard substrates at 40% extrusion moisture, however, probably reduced the loss of amino acids through the Maillard reaction (Björck & Asp, 1983), which improved IVPD. This improvement in IVPD was significant ($p \leq 0.05$) in the composite extrudates.

Table 4.3.5 Effects of extrusion cooking in-barrel moisture and of compositing on the nitrogen solubility index (NSI) of decorticated sorghum flour and whole grain bambara groundnut flour

Characteristic	**Extrusion cooking treatment**	**100% Decorticated sorghum**	**50:50 Sorghum and bambara groundnut composite**	**100% Whole grain bambara groundnuts**	**Effect of in-barrel moisture contents[3]**
NSI_1 (g/100 g protein)[1]	Not extruded	7.39 ± 1.80	43.9 ± 1.69	61.3 ± 1.53	37.5 ± 24.7
	24 % in-barrel moisture	3.0^{a2} ± 0.23	4.92^{b} ± 0.31	5.25^{b} ± 0.32	4.40^{A} ± 1.05
Flour*moisture interaction p = 0.0006	40 % in-barrel moisture	3.23^{a} ± 0.25	4.71^{b} ± 0.13	5.91^{c} ± 0.80	4.62^{A} ± 1.23
Effect of flour composition[4]		3.13^{X} ± 0.25	4.81^{Y} ± 0.25	5.58^{Z} ± 0.67	

[1] Mean ± SD (n=4, duplicate samples from 2 extrusion runs)
[2] Mean values with different lowercase superscript letters in a block differ significantly ($p<0.05$) (Flour*moisture interaction p-values stated under descriptor)
[3] Mean values with different uppercase superscript letters in a column differ significantly ($p<0.05$)
[4] Mean values with different uppercase superscript letters in a row differ significantly ($p<0.05$)

Significant increases ($p \leq 0.05$) in overall IVPD (Table 4.3.4) and NSI (Table 4.3.5) were found with the inclusion of bambara groundnuts. The increases in NSI were similar to findings with soy and sweet potato flour (Iwe et al., 2001) and sorghum and cowpea flour composites (Pelembe et al., 2002). The more soluble nature of bambara groundnut proteins consisting predominantly of globulins and albumins (Yagoub & Abdalla, 2007), compared to the insoluble sorghum kafirins (Serna-Saldivar & Rooney, 1995), presumably explained the increased NSI with bambara groundnut inclusion. Hamaker et al. (1986) described a possible link between protein solubility and digestibility in sorghum proteins. More hydrophobic proteins are thought to be less accessible to proteolytic enzymes because enzymes function in an aqueous environment. It has also been suggested that globular proteins could interfere with the cross-linking in sorghum kafirins which, in turn, alleviates its low digestibility (Dovi, 2013).

4.3.1.5 Mineral contents and Caco-2 cell uptake

The inclusion of bambara groundnut flour with sorghum flour improved zinc (32%), calcium (52%), phosphorus (14%) and magnesium (11%) contents in the composite flour significantly ($p \leq 0.05$) (Table 4.3.6). This is due to the larger content of these minerals in bambara groundnuts (Yao et al., 2015) when compared to sorghum (USDA, 2015). Minerals are heat stable and unlikely to decrease during extrusion cooking, however, increases in minerals such as iron is possible due to wear of ferrous parts of the extrusion cooker (Singh et al., 2007). The extrudate flours did, however, not contain any chromium (results not shown), the main element found in the metals of the extrusion cooker barrel and screws (K.-H. Tietz, 2015, personal communication, 17 March), which suggested that extrusion cooking did not result in contaminate minerals. Based on the dietary reference intakes of minerals for children 2-5 years of age as shown in Table 4.3.6, a 50 g dry base portion size of the composite could contribute approximately 16% iron, 14% zinc, 3% calcium, 35% phosphorus, 128% magnesium, 10% copper and 49% manganese to the required intake. The composite can, therefore, increase the mineral intake of children when compared to decorticated sorghum alone and can contribute substantially to the magnesium and manganese daily requirements.

Table 4.3.6 Mineral compositions of raw decorticated sorghum flour and whole grain bambara groundnut flour (mg/100 g, db)[1] and the dietary reference intakes (DRI) of minerals for children between ages 2-5

Flour[1]	**Iron**	**Zinc**	**Calcium[1]**	**Phosphorus**	**Magnesium**	**Copper**	**Manganese**
100% Decorticated sorghum	$4.05^{a2} \pm 0.20$	$1.95^{a} \pm 0.15$	$25.0^{a} \pm 4.31$	$293^{a} \pm 2$	$147^{a} \pm 1.97$	None detected[a]	$1.27^{b} \pm 0.03$
50:50 Sorghum and bambara groundnut composite	$3.98^{a} \pm 0.18$	$2.58^{b} \pm 0.08$	$37.9^{b} \pm 4.09$	$333^{b} \pm 3$	$163^{b} \pm 4.39$	$0.08^{b} \pm 0.02$	$1.31^{b} \pm 0.03$
100% Whole grain bambara groundnuts	$6.32^{b} \pm 0.07$	$3.04^{c} \pm 0.12$	$52.9^{c} \pm 0.70$	$411^{c} \pm 20$	$181^{c} \pm 6.79$	$0.29^{c} \pm 0.04$	$1.03^{a} \pm 0.06$
Mineral DRI (mg/day)	11.6-12.6[3]*	8.3-9.6[3]*	500-600[3]	460-500[4]	60-67[3]	0.34-0.44[5]	1.2-1.5[5]

[1] Mean ± SD (n=3)
[2] Mean values with different lowercase superscript letters in a column differ significantly ($p < 0.05$)
[3] Recommended nutrient intake from WHO/FAO (2004)
[4] RDA from the Institute of Medicine (1997)
[5] RDA (Cu) and AI (Mn) from the Institute of Medicine (2001)
* At low bioavailability

Overall, extrusion cooking at both in-barrel moistures and the inclusion of bambara groundnuts did not affect Caco-2 cell iron uptake ($p>0.05$) (Table 4.3.7). Even though phytate was slightly reduced by extrusion cooking (Table 4.3.3), phytate:iron molar ratios of all extrudates were still much higher than 1 (19 to 31, results not shown), which is the molar ratio above which iron absorption is seriously impaired (Ma et al., 2007). Differences in iron uptake with extrusion moisture and flour composition were not evident, probably due to the strong inhibitory effect phytate had on iron absorption in all treatments.

Extrusion cooking increased the overall zinc uptake (22 to 25% increase), but there were no significant effects ($p>0.05$) with extrusion moisture (Table 4.3.11). It was noticeable that extrusion cooking caused a larger increase in zinc uptake for the sorghum flours (84%) when compared to composite (8%) and bambara groundnut flours (11%). Sorghum extrudates also had the highest overall zinc uptakes and were significantly higher ($p\leq0.05$) than the composite extrudates. Phytate:zinc ratios were 50 to 86 (results not shown), i.e. above 15 for all samples, the molar ratio above which zinc absorption is seriously impaired by phytate (Ma et al., 2007). The phytate x calcium/zinc (Phy x Ca/Zn) ratio is a more helpful molar ratio to estimate zinc availability, due to the possible formation of stable zinc-calcium-phytate complexes (Fordyce et al., 1987). These complexes are believed to be an important mechanism by which phytate reduces zinc bioavailability, as zinc-calcium-phytate complexes are even stronger than phytate-zinc complexes (Ma et al., 2007). In all flours, the Phy x Ca/Zn ratios were below the critical molar ratio (200) above which zinc absorption is seriously impaired (Ma et al., 2007) (Table 4.3.7). The low ratios could explain why significant differences in zinc uptake ($p\leq0.05$) between flour compositions were found. Due to the low Phy x Ca/Zn molar ratio, a difference in phytate, calcium and zinc contents could have influenced zinc uptake significantly. Zinc uptake decreased as bambara groundnuts were included which was consistent with the increase in Phy x Ca/Zn ratios (Table 4.3.7).

Despite the overall high zinc uptake of sorghum, the low zinc content in the flour (Table 4.3.10) still caused the amount of zinc that will be taken up from the sorghum extrudates (0.07 mg zinc/100 g extrudate), to be slightly lower than from the composite extrudates (0.08 mg zinc/100 g extrudate).

Table 4.3.7 Effects of extrusion cooking in-barrel moisture and of compositing on the Caco-2 cell iron and zinc uptake and on the phytate x calcium/zinc molar ratios of decorticated sorghum flour and whole grain bambara groundnut flour

Characteristic	Extrusion cooking treatment	100% Decorticated sorghum	50:50 Sorghum and bambara groundnut composite	100% Whole grain bambara groundnuts	Effect of in-barrel moisture contents[3]
Iron uptake (%)[1]	Not extruded	5.81 ± 0.62	4.65 ± 0.35	5.56 ± 0.68	5.43 ± 0.73
	24 % in-barrel moisture	5.42 ± 0.77	5.64 ± 0.73	5.25 ± 0.54	5.44^{A} ± 0.64
Flour*moisture interaction p = 0.7014	40 % in-barrel moisture	5.18 ± 0.44	4.90 ± 0.44	4.88 ± 0.69	4.88^{A} ± 0.69
Effect of flour composition[4]		5.30^{X} ± 0.59	5.27^{X} ± 0.68	5.09^{X} ± 0.59	
Zinc uptake (%)[1]	Not extruded	1.98 ± 0.31	3.23 ± 0.52	2.97 ± 0.45	2.75 ± 0.70
	24 % in-barrel moisture	3.65 ± 0.20	3.03 ± 0.39	3.31 ± 0.41	3.35^{A} ± 0.41
Flour*moisture interaction p = 0.1410	40 % in-barrel moisture	3.56 ± 0.28	3.49 ± 0.34	3.31 ±0.36	3.45^{A} ± 0.32
Effect of flour composition[4]		3.61^{Y} ± 0.23	3.28^{X} ± 0.42	3.31^{XY} ± 0.37	
Phytate x Calcium/Zinc molar ratio	Not extruded	39.0	53.0	66.7	
	Extruded	35.6	55.6	65.3	

[1] Mean ± SD (n=4)
[2] Mean values with different lowercase superscript letters in a block differ significantly (p≤0.05) (Flour*moisture interaction p-values stated under descriptor)
[3] Mean values with different uppercase superscript letters in a column differ significantly (p≤0.05)
[4]Mean values with different uppercase superscript letters in a row differ significantly (p≤0.05)

4.3.2 Physical properties

As the food melt exits the extrusion cooker die, internal moisture flash-off due to the pressure drop from inside to outside the barrel causes an expansion of the food melt (Moraru & Kokini, 2003). The bubble structure formed is retained because the food melt cools rapidly (Pai et al., 2009). In-barrel extrusion moisture impacted on extrudate structure in the sorghum and composite extrudates. A decrease in air cell size (Fig. 4.3.1: B, a-d) and extrudate size (Fig. 4.3.1: B and C, a-d) occurred with an increase in extrusion moisture. The reduced air cell and extrudate volume corresponded with significantly reduced ($p \leq 0.05$) extrudate expansion ratio (Table 4.3.8) and significantly increased ($p \leq 0.05$) extrudate density (Table 4.3.9). Lower viscosity in the barrel at high extrusion moisture probably caused less pressure build-up in the barrel compared to lower extrusion moisture as described by Bhattacharya and Hanna (1987). A lower pressure drop from barrel to atmosphere probably caused less moisture flash off, which leads to smaller expansion (Day & Swanson, 2013). Additionally, reduced visco-elasticity of the melt in the die due to high moisture conditions probably inhibited the food melt to stretch into an expanded structure for the growth of large air cells, as suggested by Lazou and Krokida (2010b).

Extrudate expansion ratio decreased as protein and fibre contents of the flours increased with the inclusion of bambara groundnuts at 24 % in-barrel extrusion moisture (Table 4.3.8). This trend was accompanied by increased extrudate density (Table 4.3.9) and reduced air cell size cross-sectionally (Fig 3.4.1: B, a, c, e). These trends were similar to research on maize-lentil extrudates (Lazou & Krokida, 2010b). The expansion ratio of the composite extruded at 25% extrusion moisture was, however, not significantly different ($p > 0.05$) from sorghum extruded at the same moisture. Expansion is generally believed to be dependent on starch gelatinisation and the starch melt at the die (Colonna et al., 1989). A dilution of starch due to any other macromolecules would, therefore, be expected to reduce expansion, causing a denser extrudate. The reduction in size and increase in the number of air cells cross-sectionally was possibly due to increased fibre content with the inclusion of bambara groundnuts (Table 4.3.1). Devi et al. (2013) proposed that a similar phenomenon observed with the inclusion of soya with sorghum-based flours was due to fibre particles which caused more nucleation sites for air cells to develop. They also suggested that the foaming capacity of the legume supported the retention of many air cells.

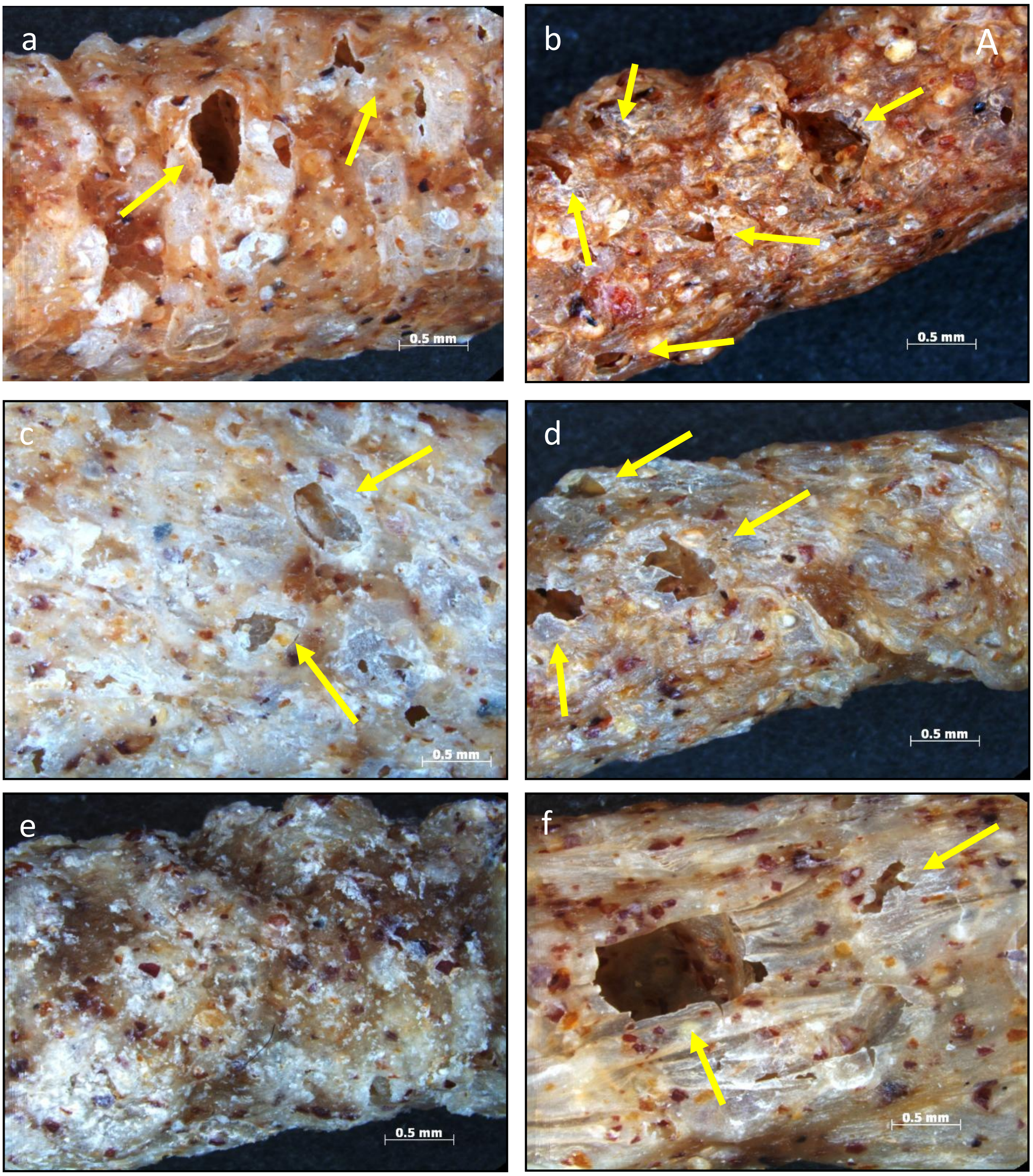


Figure 4.3.1 Stereomicroscopy of extrudates:

A Surface images, **B** Cross-sectional images, **C** Longitudinal images

a) Sorghum extruded at 24% in-barrel moisture, b) sorghum extruded at 40% in-barrel moisture, c) composite extruded at 24% in-barrel moisture, d) composite extruded at 40% in-barrel moisture, e) bambara groundnuts extruded at 24% in-barrel moisture, f) bambara groundnuts extruded at 40% in-barrel moisture.

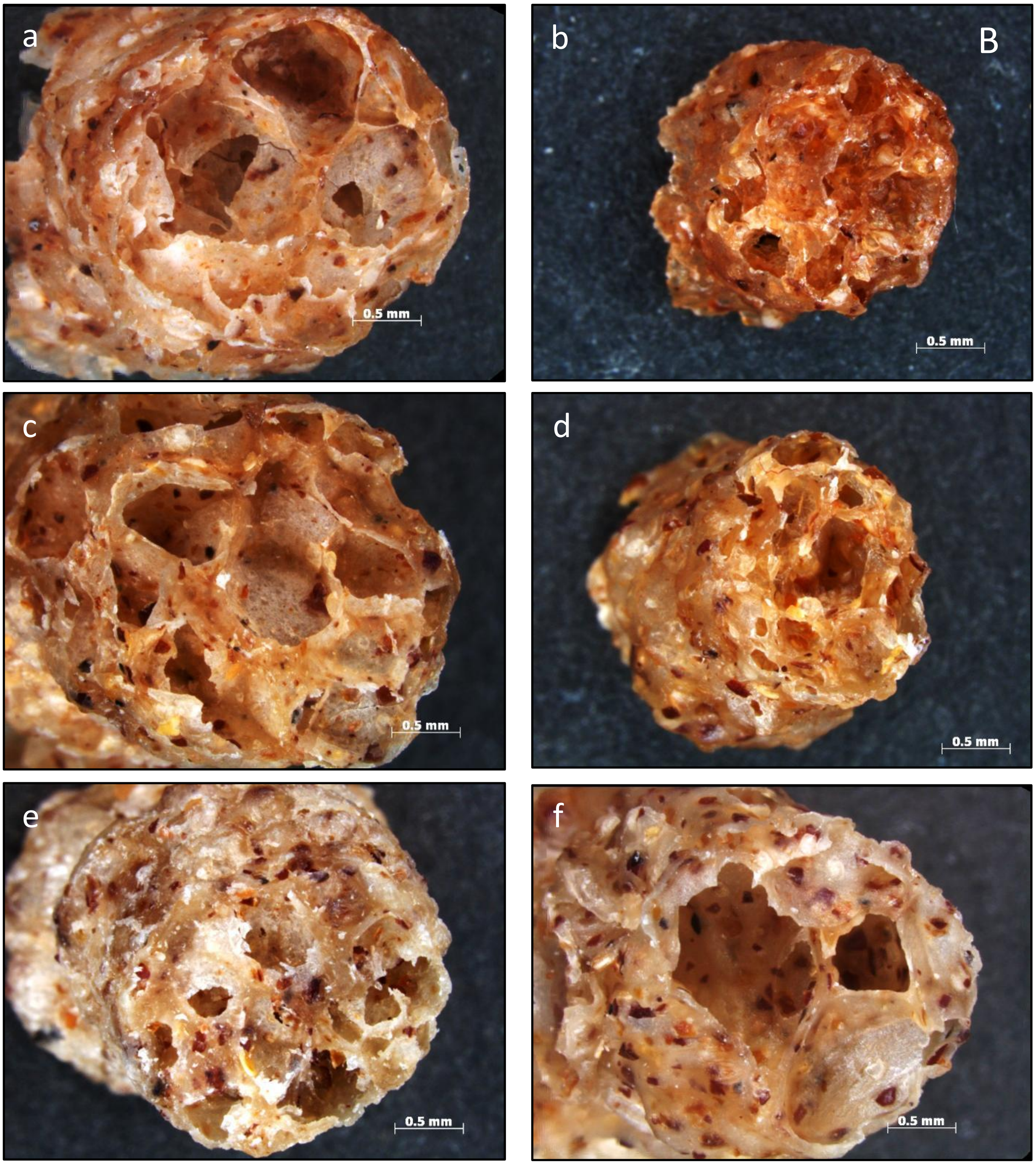


Figure 4.3.1 Continued

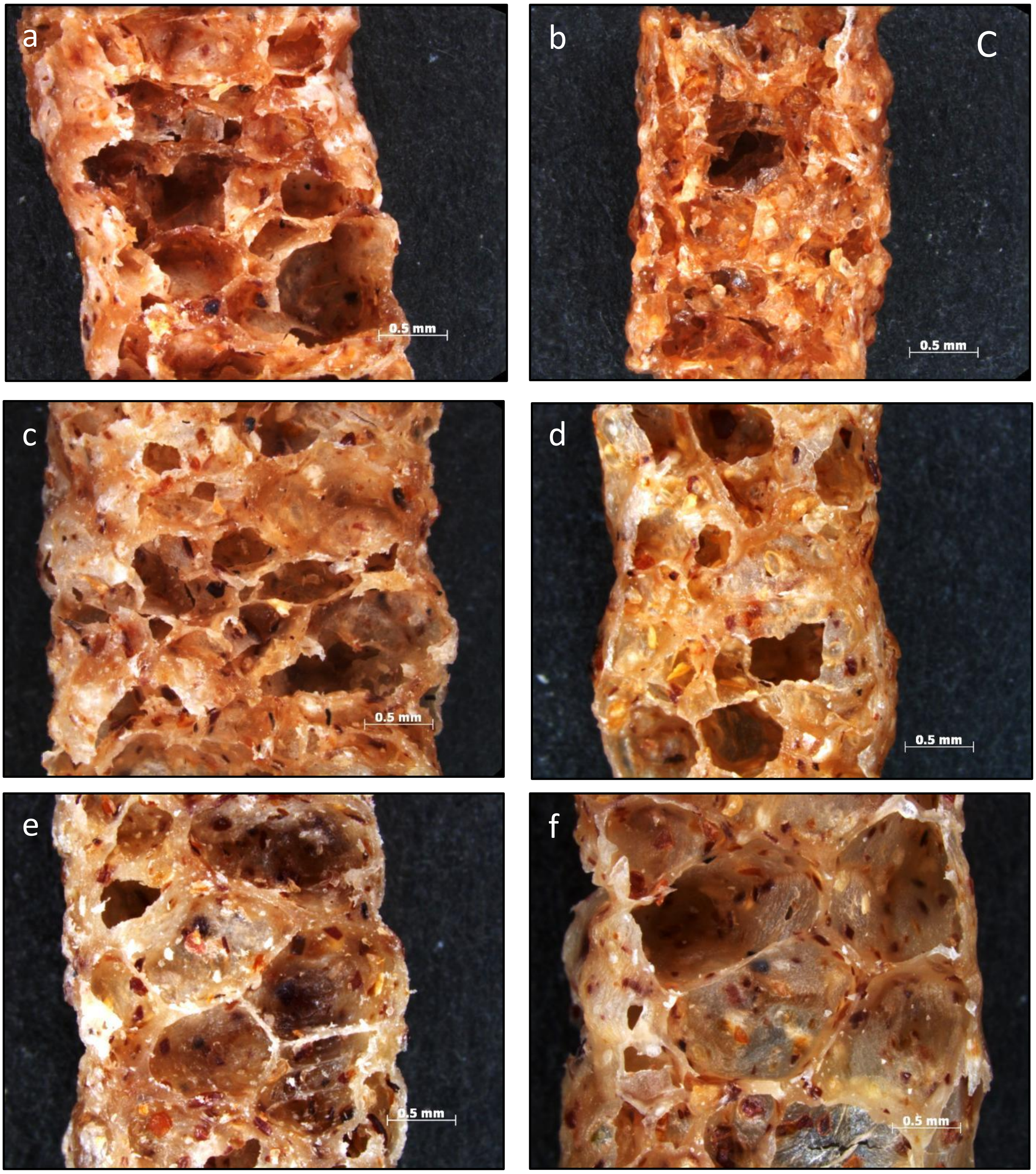


Figure 4.3.1 Continued

Table 4.3.8 Effects of extrusion cooking in-barrel moisture and of compositing on the extrudate expansion ratio of decorticated sorghum and whole grain bambara groundnut extrudates

Characteristic	Extrusion cooking treatment	100% Decorticated sorghum	50:50 Sorghum and bambara groundnut composite	100% Whole grain bambara groundnuts	Effect of in-barrel moisture content[3]
Extrudate expansion ratio ($d_{extrudate}/d_{die}$)[1]	24 % in-barrel moisture	$2.95^{cd2} \pm 0.17$	$2.73^{bc} \pm 0.25$	$2.62^{b} \pm 0.15$	$2.77^{B} \pm 0.23$
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	$2.27^{a} \pm 0.09$	$2.30^{a} \pm 0.16$	$2.98^{d} \pm 0.38$	$2.52^{A} \pm 0.41$
Effect of flour[4] composition		$2.61^{X} \pm 0.37$	$2.51^{X} \pm 0.30$	$2.80^{Y} \pm 0.34$	

[1] Mean ± SD (n=18)
[2] Mean values with different lowercase superscript letters in a block differ significantly ($p \leq 0.05$) (Flour*moisture interaction p-values stated under descriptor)
[3] Mean values with different uppercase superscript letters in a column differ significantly ($p < 0.05$)
[4] Mean values with different uppercase superscript letters in a row differ significantly ($p < 0.05$)

Table 4.3.9 Effects of extrusion cooking in-barrel moisture and of compositing on the extrudate density of decorticated sorghum and whole grain bambara groundnut extrudates

Characteristic	**Extrusion cooking treatment**	**100% Decorticated sorghum**	**50:50 Sorghum and bambara groundnut composite**	**100% Whole grain bambara groundnuts**	**Effect of in-barrel moisture content[3]**
Extrudate density (kg/m^3)[1]	24 % in-barrel moisture	$2.99^{b2} \pm 0.30$	$2.99^{b} \pm 0.43$	$5.50^{d} \pm 0.57$	$3.94^{B} \pm 1.31$
	40 % in-barrel moisture	$4.19^{c} \pm 0.43$	$3.87^{c} \pm 0.49$	$2.32^{a} \pm 0.50$	$3.42^{A} \pm 0.96$
Flour*moisture interaction p = 0.0000					
Effect of flour[4] composition		$3.61^{X} \pm 0.72$	$3.46^{X} \pm 0.64$	$3.91^{Y} \pm 1.70$	

[1] Mean ± SD (n=18)
[2] Mean values with different lowercase superscript letters differ significantly ($p \leq 0.05$) (Flour*moisture interaction p-values stated under descriptor)
[3] Mean values with different uppercase superscript letters in a column differ significantly ($p < 0.05$)
[4] Mean values with different uppercase superscript letters in a row differ significantly ($p < 0.05$)

There was, however, an opposite trend for bambara groundnuts extruded at 40% in-barrel extrusion moisture. Air cell and extrudate volume (Fig. 4.3.1: B and C, b, d, e, f), together with extrudate expansion ratio ($p \leq 0.05$) (Table 4.3.8), increased at higher extrusion moisture and with bambara groundnut inclusion. The significant decrease ($p \leq 0.05$) in extrudate density at high extrusion moisture and the inclusion of bambara groundnuts, was in agreement. The increase in air cell size at high extrusion moisture was not in the radial direction but in a longitudinal direction (Fig. 4.3.1:C, c-f). It is possible that the bambara groundnut proteins became fibrous in structure at 40% extrusion moisture, similar to what happens in the high moisture extrusion of high protein flours to produce meat analogues (Yao et al., 2004).. Table 4.2.3 shows that one of the extrusion cooking replicates for 100% whole grain bambara groundnut extruded at 40% in-barrel extrusion moisture had a higher feed rate during processing, which possibly contributed to increased expansion as explained by Ding et al. (2006). This was confirmed by the rather high standard deviation in extrudate expansion ratio and density of this treatment. In pea-wheat extrudates, an increase in air cell size was considered to be due to coalescence at higher moisture conditions (Zarzycki et al., 2015). In the same study, however, the increase in air cell size with increased extrusion moisture was accompanied by thicker air cell walls, which probably contributed to a larger extrudate density.

All extrudates displayed a ridged surface. More ruptured bubbles (indicated with yellow arrows) appeared on the surface of extrudates at the higher in-barrel extrusion moisture (Fig. 4.3.1: A). Lue and Huff (1991) proposed that it is caused when the stretching rate of the surface layer polymers is too high as food melt leaves the die. This, in turn, causes the surface layer to fail due to a disruption in this layer caused by fibre particles or by inelastic polymers at high extrusion moisture. On the surface of bambara groundnut extrudates at the 40% in-barrel moisture, layering and stretching effects of polymers appeared to develop (Fig 4.3.1: A, f), as happens in high protein and high moisture extrusion systems (Rzedzicki & Fornal, 1998).

4.3.3 Functional properties

Extrusion cooking greatly increased the WAI of all the flours (Table 4.3.10). WAI were similar to previous studies on extruded sorghum and legumes (Pelembe et al., 2002; Batista et al., 2010a). This increase was probably due to some gelatinisation of the starches which would increase the availability of hydrophilic groups in the food system (Anderson et al., 1969) and due to the increased gel-forming capacities of other macromolecules, such as dietary fibre and proteins (Anderson et al., 1969; Gomez & Aguilera, 1983). The presumed pregelatinised state of the extrudate flours was confirmed by the increase in initial paste viscosity (IV) caused by extrusion cooking (Table 4.3.11, Fig. 4.3.2) as explained by Bouvier and Clextral (2001). Extrusion cooking also caused a decrease in peak (PV), trough (TV) and final (FV) paste viscosities. This was expected and previously found in the extrusion cooking of sorghum (Mahasukhonthachat et al., 2010). The decrease in native starch due to gelatinisation during extrusion cooking, inhibits increases in viscosity caused by swelling and recrystallization of native starch during pasting (Adegunwa et al., 2014).

Overall, the 24% in-barrel extrusion moisture resulted in the extruded flours' significant lower ($p \leq 0.05$) WAI (Table 4.3.10). The severe cooking conditions at low extrusion moisture caused by high friction, pressure and heat (Pham & Del Rosario, 1984), causes a breakdown in starch polymers, especially amylose, i.e. dextrinization (Colonna et al., 1989). Dextrinization probably rendered more starch particles soluble and reduced the number of starch polymers available to absorb water. This was possibly confirmed by the significant ($p \leq 0.05$) increase in WSI at low extrusion moisture (Table 4.3.10). The lower WSI at high extrusion moisture corresponded with trends in previous research on extruded cereal-legume composites (Gujska & Khan, 1991; Hernández-Díaz et al., 2007; Lazou & Krokida, 2010a) and is probably because of less starch degradation during extrusion, caused by the lubricating effect of high moisture (Guy, 2001). Overall, the 40% in-barrel extrusion moisture produced extrudates with significantly higher ($p \leq 0.05$) PV, TV and FV (Table 4.3.11). The higher PV and FV are probably indicative of more intact starch polymers with the ability to absorb and hold water (Nnam, 2001), and to recrystallize (Al-Rabadi et al., 2011), respectively. The gelatinisation and retrogradation of starch polymers probably cause the increase in viscosities at PV and FV peaks, respectively (Mahasukhonthachat et al., 2010), and will occur at a lesser extent where starch polymers have been dextrinised. These higher pasting viscosities are also indicative of the lubricating effect of high moisture, which probably protected extrusion melt from starch dextrinization.

Table 4.3.10 Effects of extrusion cooking in-barrel moisture and of compositing on the water absorption index (WAI) and water solubility index (WSI) of decorticated sorghum flour and whole grain bambara groundnut flour

Characteristic	Extrusion cooking treatment	100% Decorticated sorghum	50:50 Sorghum and bambara groundnut composite	100% Whole grain bambara groundnuts	Effect of in-barrel moisture contents[3]
WAI (g/g sample, db)[1]	Not extruded	2.66 ± 0.06	2.79 ± 0.01	2.33 ± 0.28	2.59 ± 0.25
	24 % in-barrel moisture	6.55^{b2} ± 0.11	6.98^{c} ± 0.17	4.60^{a} ± 0.45	6.05^{A} ± 1.08
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	6.71^{bc} ± 0.17	6.81^{bc} ± 0.13	6.61^{b} ± 0.88	6.71^{B} ± 0.52
Effect of flour composition[4]		6.63^{Y} ± 0.16	6.89^{Z} ± 0.17	5.60^{X} ± 1.18	
WSI (g/100 g sample, db)[1]	Not extruded	5.07 ± 0.28	20.27 ± 1.42	32.66 ± 0.97	19.33 ± 12.39
	24 % in-barrel moisture	3.33^{a2} ± 0.17	10.92^{c} ± 1.08	25.02^{e} ± 2.75	13.08^{B} ± 9.26
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	3.26^{a} ± 0.20	9.39^{b} ± 1.74	14.31^{d} ± 1.16	8.46^{A} ± 4.67
Effect of flour composition[4]		3.26^{X} ± 0.20	9.39^{Y} ± 1.74	19.66^{Z} ± 5.85	

[1] Mean ± SD (n=6, triplicate samples from 2 extrusion runs)

[2] Mean values with different lowercase superscript letters in a block differ significantly (p≤0.05) (Flour*moisture interaction p-values stated under descriptor)

[3] Mean values with different uppercase superscript letters in a column differ significantly (p<0.05)

[4] Mean values with different uppercase superscript letters in a row differ significantly (p<0.05)

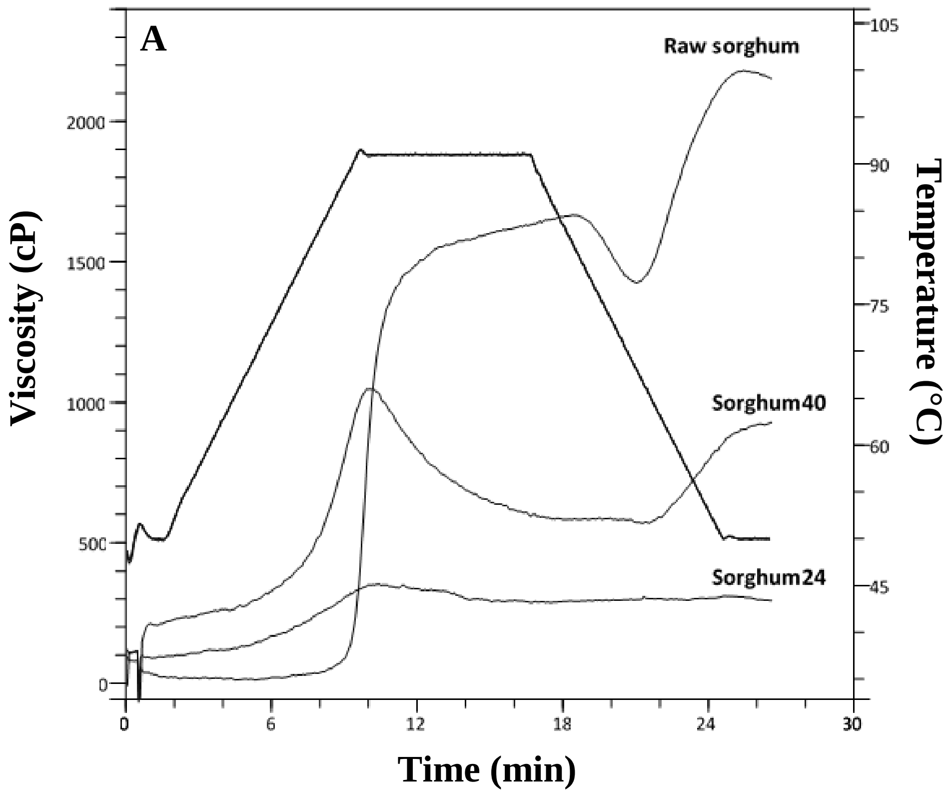
Table 4.3.11 Effects of extrusion cooking in-barrel moisture and of compositing on the initial, peak viscosity, trough and final viscosities of decorticated sorghum and whole grain bambara groundnut extrudates

Characteristic	Extrusion cooking treatment	100% Decorticated sorghum	50:50 Sorghum and bambara groundnut composite	100% Whole grain bambara groundnuts	Effect of in-barrel moisture content[3]
Initial viscosity (cP)[1]	Not extruded	33.1 ± 0.0	22.7 ± 26.3	15.2 ± 3.4	23.7 ± 13.1
	24 % in-barrel moisture	83.7^{a2} ± 8.3	121.1^{b} ± 4.4	158.7^{c} ± 13.7	121.1^{A} ± 34.7
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	182.4^{d} ± 22.8	101.8^{ab} ± 13.0	98.8^{ab} ± 18.0	127.6^{A} ± 43.7
Effect of flour composition[4]		133.0^{Y} ± 55.1	110.0^{X} ± 14.0	128.7^{Y} ± 35.2	
Peak viscosity (cP)[1]	Not extruded	1670.2 ± 34.4	942.1 ± 1.0	NA[5]	1378.9 ± 399.5
	24 % in-barrel moisture	350.6 ± 20.3	210.2 ± 17.9	NA	290.5^{A} ± 77.2
	40 % in-barrel moisture	1171.8 ± 164.8	603.7 ± 22.8	262.0 ± 25.7	679.1^{B} ± 401.7
Effect of flour composition[4]		761.3^{Y} ± 452.1	435.0^{X} ± 211.2	262.0^{X} ± 25.7	

Table 4.3.11 Continued

Characteristic	**Extrusion cooking treatment**	**100% Decorticated sorghum**	**50:50 Sorghum and bambara groundnut composite**	**100% Whole grain bambara groundnuts**	**Effect of in-barrel moisture content[3]**
Trough viscosity (cP)[1]	Not extruded	1437.4 ± 34.4	906.4 ± 47.9	NA	1171.9 ± 293.2
	24 % in-barrel moisture	269.9^{c2} ± 14.8	145.5^{ab} ± 2.6	85.9^{a} ± 7.5	169.8^{A} ± 87.0
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	611.4^{d} ± 69.0	170.6^{b} ± 14.0	95.3^{a} ± 2.0	292.4^{B} ± 240.6
Effect of flour composition[4]		465.0^{Z} ± 189.1	162.2^{Y} ± 17.0	91.3^{X} ± 6.8	
Final viscosity (cP)[1]	Not extruded	2184.6 ± 40.6	1435.7 ± 53.3	1254.6 ± 45.0	1625.0 ± 428.9
	24 % in-barrel moisture	299.1^{bc2} ± 3.9	163.9^{a} ± 33.6	165.5^{a} ± 25.2	213.7^{A} ± 70.8
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	1073.9^{d} ± 199.3	310.8^{c} ± 3.7	194.8^{abc} ± 9.0	526.5^{B} ± 420.4
Effect of flour composition[4]		686.5^{Y} ± 434.2	247.9^{X} ± 80.9	180.2^{X} ± 23.5	

[1] Mean ± SD (n=4, duplicate samples from 2 extrusion runs)
[2] Mean values with different lowercase superscript letters in a block differ significantly ($p \leq 0.05$) (Flour*moisture p-values stated under descriptor)
[3] Mean values with different uppercase superscript letters in a column differ significantly ($p < 0.05$)
[4] Mean values with different uppercase superscript letters in a row differ significantly ($p < 0.05$)
[5] NA – Not applicable – no peak or trough viscosity formed in pasting curves of respective flours



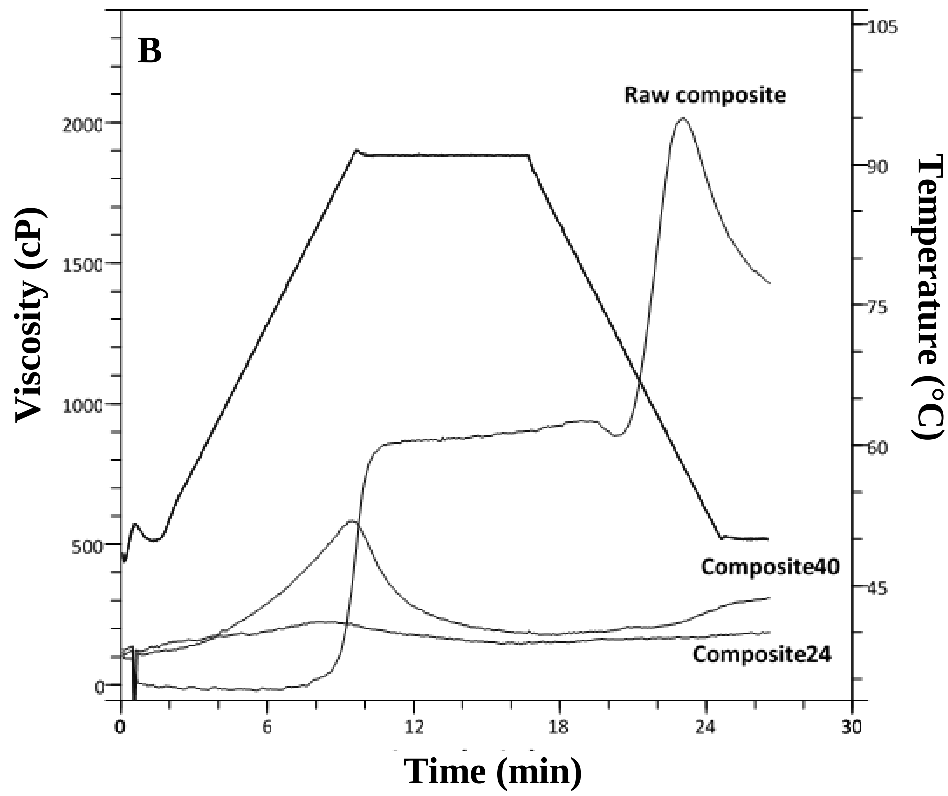


Figure 4.3.2 Effect of extrusion cooking in-barrel moisture on the pasting curves of: **A** Sorghum flours: Raw and extruded at 24% (Sorghum24) and 40% (Sorghum40) in-barrel moisture, respectively. **B** Composite flours: Raw and extruded at 24% (Composite24) and 40% (Composite40) in-barrel moisture, respectively. **C** Bambara groundnut flours: Raw and extruded at 24% (Bam24) and 40% (Bam40) in-barrel moisture, respectively

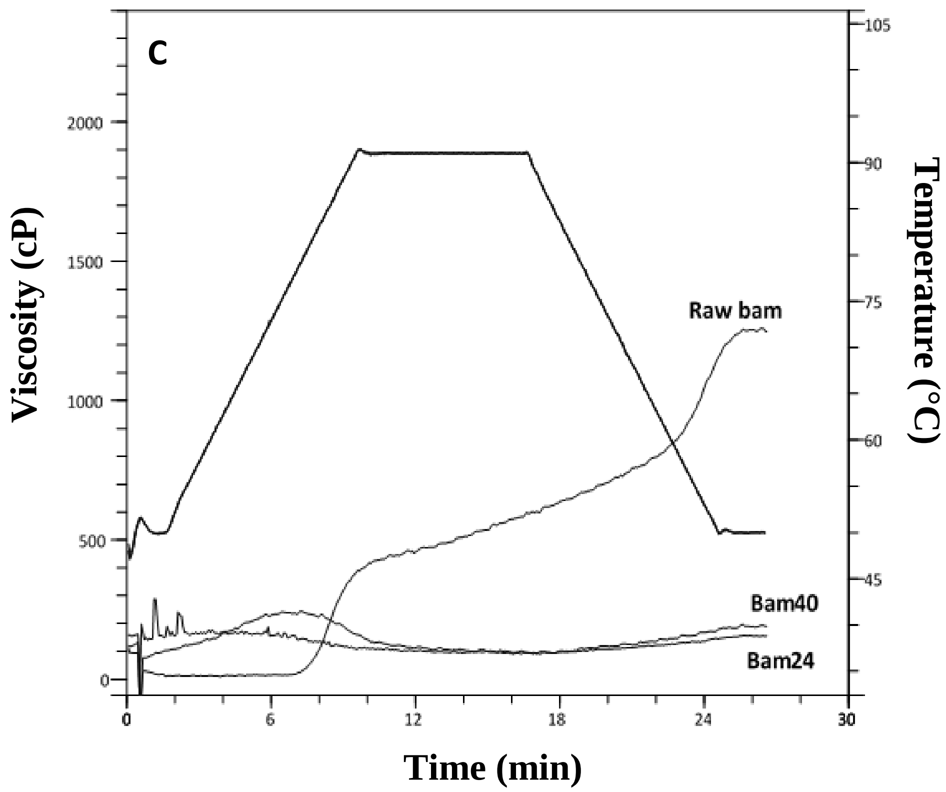


Figure 4.3.2 Continued

The higher WAI at higher extrusion moisture was, however, only significant ($p \leq 0.05$) in 100% bambara groundnut extrudate flours (Table 4.3.10). A higher amylose content is believed to yield higher WAI (Mercier & Feillet, 1975). The higher amylose content of bambara groundnuts (Ashogbon, 2014), when compared to sorghum (Beta & Corke, 2001), could have caused that the effect of extrusion moisture on WAI was augmented in bambara groundnut extrudates. The higher in bambara groundnut WAI at higher extrusion moisture was similar to trends found for extruded garbanzo and pinto beans (Gujska & Khan, 1991).

The extrudates had red and yellow colour components (Table 4.3.6). Significantly higher ($p \leq 0.05$) red and yellow values were obtained at 40% in-barrel extrusion moisture. The plasticizing effect of high moisture extrusion cooking probably inhibited Maillard browning or colour pigment degradation, which are believed to be prevalent at severe processing conditions (Chen et al., 1991).

Table 4.3.12 Effects of extrusion cooking in-barrel moisture and of compositing on the L, a and b colour components of decorticated sorghum flour and whole grain bambara groundnut flour after pasting

Characteristic	Extrusion cooking treatment	100% Decorticated sorghum	50:50 Sorghum and bambara groundnut composite	100% Whole grain bambara groundnuts	Effect of in-barrel moisture content[3]
L[1]	Not extruded	44.5 ± 0.9	48.4 ± 0.6	48.2 ± 1.1	47.0 ± 2.0
	24 % in-barrel moisture	$49.0^{c2} \pm 0.2$	$48.7^{c} \pm 0.8$	$46.6^{a} \pm 0.7$	$48.1^{B} \pm 1.3$
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	$47.0^{a} \pm 0.5$	$47.8^{b} \pm 0.5$	$47.7^{b} \pm 0.4$	$47.5^{A} \pm 0.6$
Effect of flour composition[4]		$48.0^{Y} \pm 1.1$	$48.2^{Y} \pm 0.8$	$47.1^{X} \pm 0.8$	
a[1]	Not extruded	1.79 ± 0.10	1.74 ± 0.14	1.18 ± 0.12	1.56 ± 0.30
	24 % in-barrel moisture	$1.05^{a2} \pm 0.10$	$1.51^{b} \pm 0.21$	$1.66^{b} \pm 0.16$	$1.43^{A} \pm 0.31$
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	$1.83^{c} \pm 0.10$	$1.52^{b} \pm 0.14$	$1.53^{b} \pm 0.32$	$1.63^{B} \pm 0.25$
Effect of flour composition[4]		$1.50^{X} \pm 0.41$	$1.52^{X} \pm 0.17$	$1.60^{X} \pm 0.25$	
b[1]	Not extruded	1.00 ± 0.10	1.23 ± 0.09	1.81 ± 0.17	1.38 ± 0.37
	24 % in-barrel moisture	$0.52^{a2} \pm 0.10$	$1.18^{b} \pm 0.13$	$0.68^{a} \pm 0.15$	$0.82^{A} \pm 0.31$
Flour*moisture interaction p = 0.0000	40 % in-barrel moisture	$1.67^{d} \pm 0.28$	$1.39^{c} \pm 0.22$	$1.48^{c} \pm 0.15$	$1.51^{B} \pm 0.24$
Effect of flour composition[4]		$1.10^{X} \pm 0.63$	$1.27^{Y} \pm 0.20$	$1.06^{X} \pm 0.44$	

[1] Mean ± SD (n=12)

[2] Mean values with different lowercase superscript letters in a block differ significantly ($p \leq 0.05$) (Flour*moisture interaction p-values stated under descriptor)

[3] Mean values with different uppercase superscript letters in a column differ significantly ($p<0.05$)

[4] Mean values with different uppercase superscript letters in a row differ significantly ($p<0.05$)

Overall, flour composition effected extrudate WAI, but the effect was only significant ($p \leq 0.05$) at the low in-barrel moisture (Table 4.3.10). Bambara groundnuts extruded at low extrusion moisture had a remarkably low WAI. The high WSI (Table 4.3.10) and low IV (Table 4.3.11 and Fig. 4.3.2) of bambara groundnut flour extruded at 24% in-barrel moisture, suggests that extreme degradation of starch polymers had taken place. The high extrusion temperatures (up to 142 °C) in zones 4 and 5 in the barrel (Table 4.2.3), were the probable cause. WSI increased substantially ($p \leq 0.05$) with bambara groundnut inclusion. Pelembe et al. (2002) attributed the same trend in sorghum-cowpea composites to the more soluble proteins present in legumes, as opposed to sorghum. A positive correlation between NSI and WSI ($r = 0.71$, $p \leq 0.05$) in this study supported this hypothesis.

Overall, the inclusion of bambara groundnut caused a significant decrease ($p \leq 0.05$) in extrudate PV, TV and FV (Table 4.3.11 and Fig. 4.3.2). This corresponded with previous studies where the addition of fermented bambara groundnuts to fermented sorghum (Nnam, 2001) and the incorporation of various legumes into wheat and sorghum (Balasubramanian et al., 2011) caused lower overall viscosity, PV and FV. The formation of starch-fat and starch-protein complexes has been suggested as a cause of a reduction in extrusion melt viscosity with the inclusion of legumes (Balasubramanian et al., 2011). The higher starch content of sorghum (Table 4.3.1) probably caused higher extrudate PV due to greater starch gelatinization as suggested by Adegunwa et al. (2014). Bambara groundnut had no clear viscosity peaks in raw flour and 24% extrusion moisture extrudate (Fig 4.3.2, C). In raw flour, the absence of a viscosity peak was probably due to the presence of proteins which restricted the complete swelling of starch granules with heating, as suggested by Mahasukhonthachat et al. (2010). The absence of a viscosity peak in the low extrusion moisture bambara groundnut extrudate flour, however, was probably as a result of the complete disintegration of starch granules and disruption of crystallinity by severe extrusion cooking conditions as suggested by Robin et al. (2015). This was also suggested by its low WAI (Table 4.3.10).

A decrease in break-down viscosity with the inclusion of bambara groundnuts was also observed. This was similar to previous research on the addition of legumes to sorghum and wheat blends (Balasubramanian et al., 2011) and was possibly due to the increased degree of organisation provided by protein-starch interactions as proposed by Olayinka, Adebowale and Olu-Owolabi (2008). In the same study, reduced setback viscosity with the addition of legumes was suggested to be due to the inhibition of starch retrogradation and hydrogen bond formation between proteins. In this present work, reduced setback viscosity with the addition of bambara groundnuts was also observed for extrusion cooking at high moisture.

The inclusion of bambara groundnuts made the extrudate pastes significantly darker ($p \leq 0.05$), rendering darker extrudates (Table 4.3.12). This was probably due to the increased level of available amino acids, in particular lysine, in bambara groundnut flour to participate in the Maillard reaction, which produce dark pigments (Ames, 1998). This trend was however only significant ($p \leq 0.05$) at low extrusion moisture where conditions are favourable for the Maillard reaction to occur (Cheftel, 1986).

4.4 CONCLUSIONS

The inclusion of whole grain bambara groundnut flour with decorticated sorghum flour increases the protein and mineral contents and improves IVPD. A serving size of 50g (db) sorghum-bambara groundnut composite extrudate could contribute approximately 81% of the daily protein requirements of children aged 2-5 years. High extrusion moisture produces higher overall IVPD and greater reductions overall in assayable phenolics. Extrusion cooking at low extrusion moisture causes greater overall reductions in phytate. Inclusion of bambara groundnuts causes reduced zinc bioaccessibility due to their higher phytate content. Low extrusion moisture and bambara groundnut inclusion reduces extrudate peak, trough and final pasting viscosities, which could increase nutrient-density when the flours are applied as porridges. Sorghum-bambara groundnut composite flour extruded at low extrusion moisture could yield nutrient-dense instant porridge flours. Extrusion cooking of composite flour at low extrusion moisture maintains high extrudate expansion. This points to the possible application of these extrudates as expanded snacks. The positive nutritional improvement and promising physical and functional qualities of composite flours extruded at low extrusion moisture produces extrudates which are potentially suitable as convenience-type products to alleviate PEM and increase mineral intake among young children aged 2-5 years.

5. GENERAL DISCUSSION

This chapter is divided into three sections. After a critical review of the experimental design, the first section considers weaknesses in the research conducted. The second section evaluates the major findings concerning the effect of flour composition and extrusion in-barrel moisture on extrudate nutritional, physical and functional properties. Models to provide an explanation for unexpected findings in the research are also proposed. The third section suggests further actions to complement the findings of the current research.

5.1 Critical review of the experimental work

The effects of bambara groundnut inclusion and in-barrel extrusion moisture on the nutritional, physical and functional properties of sorghum extrudates were determined in this study. The experimental design comprised of two levels of in-barrel extrusion moistures and two levels of bambara groundnut inclusion. The two levels of moisture applied were greatly different compared to most research involving starch-based extrusion where moisture levels are kept in the low-moisture region (see section 2.5). The application of only two extrusion moisture and bambara groundnut levels was insufficient to comprehensively investigate their effects and were, therefore, a drawback in the experimental design. The current study was, however, preliminary and was undertaken to establish the performance of bambara groundnuts in an extrusion cooker at different moisture levels, on its own and as a composite with sorghum. An industrial-scale extrusion cooker was applied for the investigation and required large amounts of flour for the production of extrudates. Investigating more extrusion moisture and bambara groundnut levels would have demanded more bambara groundnuts than were available.

If more levels of in-barrel extrusion moisture and more levels bambara groundnut inclusion were applied, response surface methodology (RSM) could be performed. RSM enables the development, improvement or optimisation of a process to yield the optimum response (Baş & Boyacı, 2007) and has been widely applied in extrusion cooking. The effects of feed moisture (12-44%) and ingredient variation of cowpea (0-20%) and groundnuts (0-10%) on physical and functional properties of sorghum extrudates have been modelled using RSM (Asare et al., 2010). The responses towards such large moisture variations were found to be mostly non-linear. In the same study, optimum feed moisture, cowpea and groundnut levels for the production of puffed snacks were calculated using RSM. Similarly, RSM has also been applied to optimise nutritional (slowly digested starch levels) and physical (expansion ratio) responses in sorghum-maize blends extruded at various temperatures and moisture levels (Licata et al., 2015). If the application of RSM was possible, optimum conditions for the desired product and the nature of interaction between the two variables could have been established. Further, a third extrusion repetition would have tested the repeatability of the treatments more accurately. As stated, protein quality can be defined by the amino acid composition of proteins and digestibility in a considered food (FAO/WHO/UNU, 1985). The Protein Digestibility Corrected Amino Acid Score (PDCAAS) is a measure of protein quality which measures the sufficiency of protein in terms of quantity and availability for a certain age group (WHO/FAO/UNU, 2007). The IVPD measured in this study gave an indication of protein availability only. The measurement of indispensable (essential) amino acids would have given direct data on protein quality. The measurement of available lysine, especially, would have been relevant as it is the limiting amino acid in cereal-based diets (WHO/FAO/UNU, 2007). Available lysine would have enabled the calculation of PDCAAS to give a specific indication of extrudate protein quality for children between 2-5 years of age who are at risk of PEM (Black et al., 2008).

5.2 Major findings

Bambara groundnut inclusion with sorghum at a 50% level was nutritionally beneficial. As discussed in section 4.3, protein and mineral contents were improved. IVPD was also improved, presumably due to the more soluble globulin-type proteins in bambara groundnuts (Poulter, 1981). Based on by WHO/FAO/UNU (2007) values, 50 g (db) sorghum-bambara groundnut composite extrudate per day can provide 81% of the protein requirement of children between 2 and 5 years of age (see section 4.3.1.4). The same portion size per day can also contribute 17% and 14% of the iron and zinc recommended nutrient intakes, respectively (see section 4.3.1.5).

Extrusion cooking at 40% in-barrel moisture seemed to have been a less severe treatment when compared to 24% in-barrel moisture. Possible indications of less dextrinized and gelatinised starch (through WSI, WAI and pasting profiles), together with higher protein digestibility and solubility of extrudates at 40% in-barrel moisture (see section 4.3), point to the mild nature of this treatment. Bhattacharya and Hanna (1987) summarised in terms of mathematical equations how an increase in extrusion moisture caused a decrease in viscosity and in pressure in the barrel, thereby reducing the amount of specific energy exerted onto the extrudate melt. It can, therefore, be expected that the amount of shear, frictional forces and heat build-up in the barrel would decrease with reduced viscosity.

There were interactions between flour composition and extruder in-barrel moisture contents in terms of some extrudate properties. The effects of the two independent variables on some properties should, therefore, be considered together and not just individually. The effect of different in-barrel moisture on WAI and NSI was greater with bambara groundnut extrudates than the sorghum and composite extrudates. WAI and NSI at low in-barrel moisture were significantly lower ($p \leq 0.05$) when compared to high in-barrel extrusion moisture of bambara groundnut extrudates (Tables 4.3.10 and 4.3.5). Extrusion moisture did, however, not have a significant effect ($p > 0.05$) on WAI and NSI in sorghum and composite extrudates.

This effect of extrusion moisture on WAI and NSI in bambara groundnut extrudates alone can be explained by the formation of starch-protein complexes, as proposed by Allen et al. (2007) working with a whey-maize flour extrusion system. The unfolding of protein molecules caused by the heat and shear in the extruder barrel possibly exposes binding sites for starch, sugar or dextrin molecules. The complexing of protein and carbohydrates presumably yields insoluble polymers, as sites usually available for water interaction are occupied by complexes. The effects probably reduce WAI and NSI (Allen et al., 2007). Such complexes are, therefore, thought to form to a great extent in bambara groundnut flour extruded at low in-barrel extrusion moisture only. Reduced sites for binding water was also indicated by the absence of a pasting viscosity peak in bambara groundnut extrudates extruded at 24% in-barrel moisture (Fig. 4.3.2 C).

There is other supporting evidence to suggest that protein-starch complexes predominantly formed at low in-barrel moisture with the bambara groundnut extrudates only. Amylose molecules especially are thought to interact with proteins, forming polymer complexes which align along the extrudate melt flow (Allen et al., 2007). The amylose content of bambara groundnut flour (approximately 37 g/100 g starch) (Ashogbon, 2014) is higher than in sorghum (approximately 28 g/100 g starch) (Beta & Corke, 2001). Bambara groundnut flour is also far richer in protein (Table 4.3.1). Bambara groundnut protein is also rich in polar and charged amino acids such as threonine, glutamic acid, lysine and arginine (Nwokolo, 1987), which would possibly enable interaction with the polar groups of amylose. Interactions between protein and amylose molecules are thought to be covalent in nature (Allen et al., 2007), but could also include hydrogen bonding, ionic interaction and some Van Der Waals forces. Sorghum kafirin protein, in contrast, is rich in non-polar amino acids such as proline, alanine and leucine (Taylor & Schüssler, 1986). As mentioned, protein-starch complexes are thought to align longitudinally with extrusion flow. This probably causes a decrease in radial expansion but favours longitudinal expansion (Allen et al., 2007). The extrudate expansion ratio of bambara groundnut extruded at 24% in-barrel moisture was low (Table 4.3.8). Extrudate structure, however, showed increased longitudinal expansion when compared with sorghum and composite extrudates, as observed by stereomicroscopy (Fig. 4.3.1C.e).

Extrusion cooking of bambara groundnuts at high in-barrel moisture probably dispersed protein and carbohydrate polymers to an extent where protein-starch complexing was limited. The high moisture level in the barrel could also have caused the binding of water molecules to available sites on amylose and protein molecules, instead of these polymers interacting with each other. This possible difference in protein-starch interaction in the barrel at low and high in-barrel moisture in the extrusion of bambara groundnuts is depicted in Figure 5.1. This proposed model also lead to the possible explanation of another unexpected trend which was found in the extrudate expansion ratio of this study and will be discussed further.

The high extrudate expansion ratio and low extrudate density for bambara groundnuts extruded at 40% in-barrel moisture relative to the trends in bambara groundnut inclusion and extrusion moisture for other extrudates, was not expected (Tables 4.3.8 and 4.3.9). As stated, expansion occurs as the extrudate melt exits the extrusion cooker die (Moraru & Kokini, 2003). Internal moisture flash-off due to the pressure drop from inside to outside the barrel causes an expansion of the food melt. A porous structure is formed due to the retention of bubbles as the food melt cools rapidly (Pai et al., 2009). High levels of protein, fat, fibre and moisture, which was the case in bambara groundnuts at high moisture, are believed to limit extrudate expansion (Moraru & Kokini, 2003), due to the dilution of gelatinised starch. Gelatinised starch forms a viscoelastic dough for an expanded structure. At high in-barrel moisture, the viscosity of the food melt would probably be reduced to an extent where the visco-elasticity of the extrusion melt is lost and where the food melt can no longer stretch into an expanded structure optimally.. The growth and retention of large air cells, as suggested by Lazou and Krokida (2010b), will subsequently be limited which leads to small expansion. Due to lowered melt viscosity, the pressure drop from inside to outside the barrel was probably also not large enough to cause good expansion, as explained by Mahasukhonthachat et al. (2010). Extrudate expansion ratio was, therefore, expected to decrease and extrudate density expected to increase with the maximum amount of bambara groundnuts and in-barrel extrusion moisture.

It is possible that the high level of amylose, protein (De Mesa et al., 2009) and fibre (Camire & King, 1991) as are present in bambara groundnut flour when compared to sorghum flour, bound more water in the extruder barrel. The hydrophilicity of globulin-type proteins in bambara groundnuts could especially contribute to water binding, as has been suggested for a starch-based system to which soy proteins were added (Faubion & Hoseney, 1982). The low level of free water in the extruder barrel could possibly have led to an increase in viscosity when compared to sorghum and composite extruded at high moisture. Della Valle et al. (1996) also suggested that the entanglement of amylose molecules with each other leads to an increase in viscosity in an extrusion cooking system. The protection of amylose, protein and fibre polymers against extensive depolymerisation by the high extrusion moisture could have rendered more of these hydrophilic polymers available to bind moisture in the barrel, when compared to sorghum extrudates, as shown in Fig 5.2. A higher viscosity of bambara groundnut extrudate melt as it approached the extruder die when compared to the sorghum and composite extrudate melts, could have provided sufficient pressure drop for high expansion, as the extrudate exited the die. Undepolymerised protein and carbohydrate polymers in bambara groundnut extruded at high in-barrel moisture could then be able to support high expansion, producing extrudates with large air cells, as observed (Figure 4.3.1 B.f and C.f.).

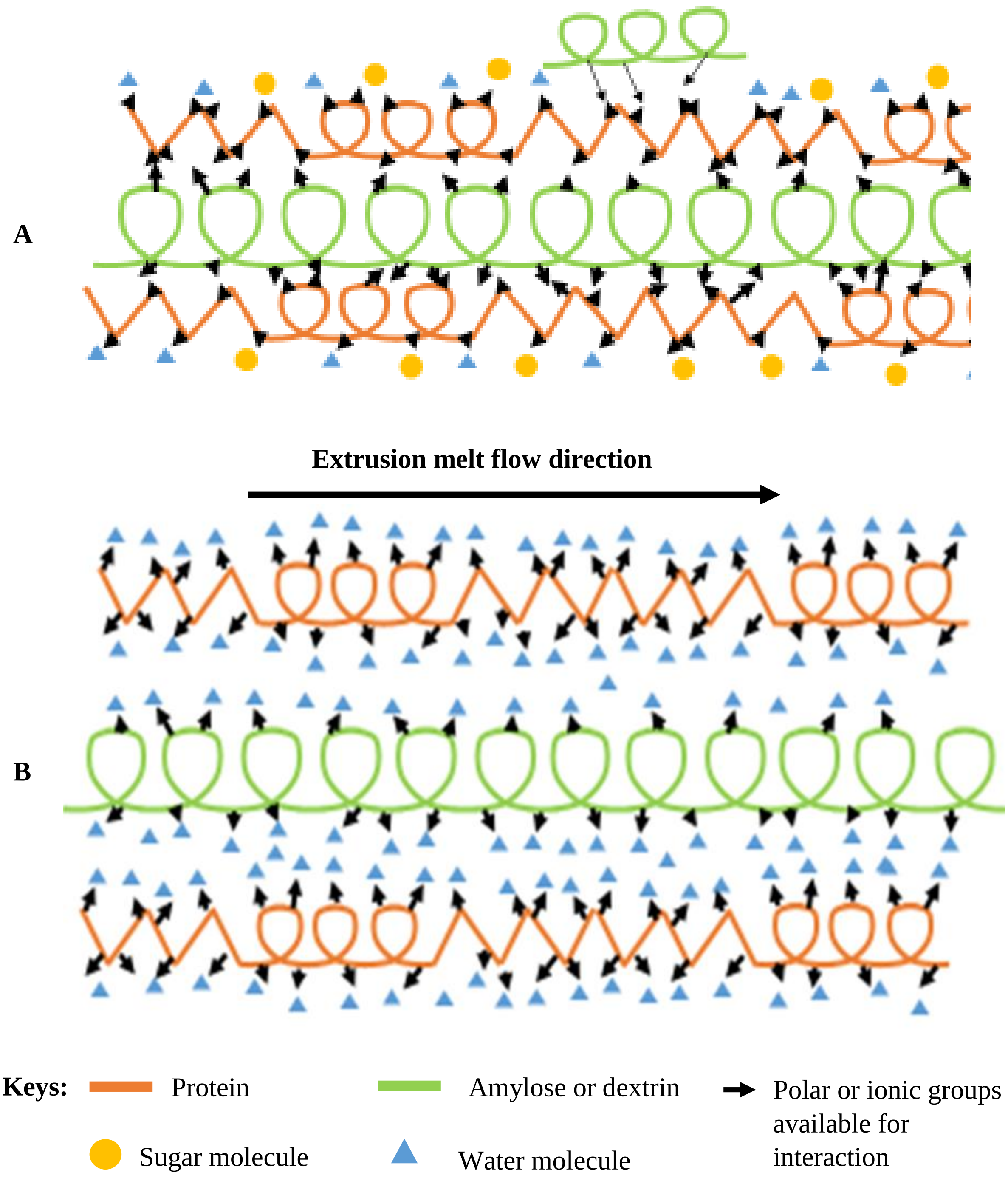


Figure 5.1 Model to explain the effect of in-barrel moisture levels on WAI, pasting curves, NSI and extrudate expansion ratio based on possible interactions between protein and carbohydrates during the extrusion cooking of bambara groundnut flour.

A Low in-barrel moisture, **B** High in-barrel moisture

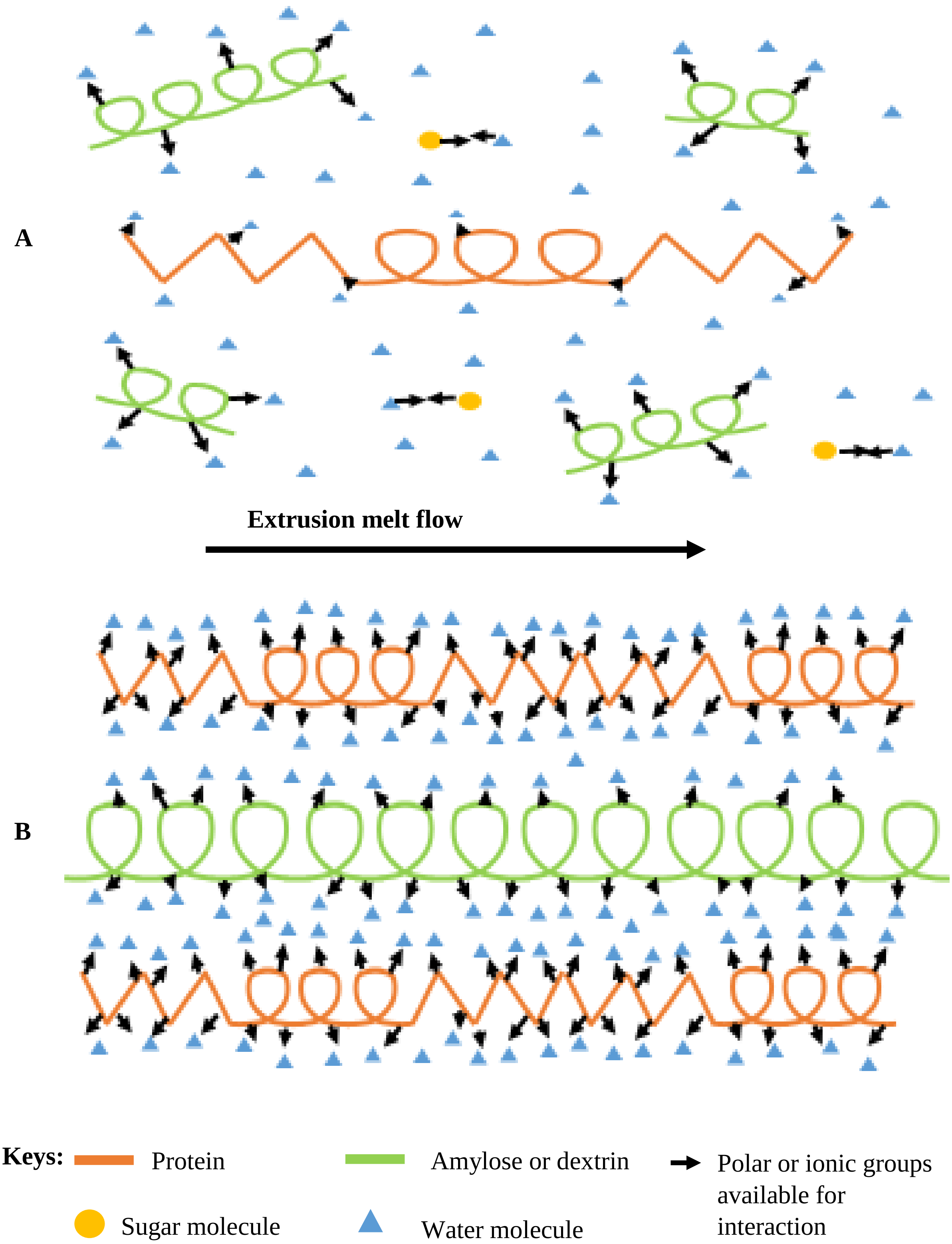


Figure 5.2 Model to explain the effect of flour composition on extrudate expansion ratio based on the binding of water molecules during extrusion cooking at high in-barrel moisture.

A Sorghum flour

B Bambara groundnut flour

5.3 Going forward

The promising nutritional, physical and functional properties of sorghum-bambara composite extrudates for application in convenience-type products such as expanded snacks or instant porridges, needs to be supported by further research. The extrudates have the potential to be applied as is, as an expanded snack, or milled, as a porridge. Experimentation with additives such as sugar, salt and flavours should be conducted to improve the extrudates' appeal to consumers. Consumer sensory testing of sorghum-bambara groundnut extrudates as an expanded snack has been evaluated (Sibandze, unpublished BSc Honours Research, University of Pretoria). Similar low moisture extrusion conditions to this present work were applied during extrusion and an oil-based barbeque flavouring was added to the extrudates. A composite ratio of 50:50 was regarded as likeable and scored a similar hedonic rating for degree of liking to a commercial barbeque flavoured expanded maize snack. The application of descriptive sensory testing to the extrudates is required to characterise the extrudates in terms of colour, texture, aroma and flavour through the perception of sight, smell, taste, touch and hearing of a trained panel (Sidel & Stone, 1993). Characterisation of extrudates could give insight into the effects of extrusion moisture level and bambara groundnut inclusion on the sensory attributes of extrudates. The inclusion of bambara groundnut probably causes a beany flavour as found by Brough et al.(1993) and increases the hardness of extrudates, because protein content is increased (Olapade & Aworh, 2012). Extrusion moisture which influenced extrudate expansion, in turn, influences sensory attributes such as hardness (Liu et al., 2000).

The nutritional, physical and functional properties of the extrudates should also be compared to similar commercial products. In the development of a composite instant African porridge from extruded sorghum and micronized cowpeas, Vilakati et al. (2015) compared its nutritional and functional properties with those of a commercial maize-soy instant flour. Flours produced from sorghum-bambara groundnut composite extrudates should be compared to a similar commercial instant flour. Sorghum-bambara groundnut composite extruded at 24% in-barrel extrusion moisture should be compared to a commercial expanded maize snack.

Costing will be required to determine the financial implications of producing extrudate products from sorghum and bambara groundnuts. An aim of the current study was to discover a means of addressing malnutrition such as PEM, which is especially prevalent among poorer communities in Africa. The economic viability of the suggested products is, therefore, important in assessing the feasibility of applying extrudates as a solution for PEM in such communities. The price of the products should compete with those of commercially available and popular products among children in these communities, otherwise, the proposed solution would be futile.

6 CONCLUSIONS AND RECOMMENDATIONS

The nutritional quality of sorghum-based extrudates is improved with the inclusion of bambara groundnuts, in terms of protein and mineral (zinc, calcium, phosphorus, magnesium) contents, and protein quality in terms of digestibility. This is because bambara groundnuts are richer in protein and minerals compared to sorghum. The soluble globulin-type proteins of bambara groundnuts are also more digestible than the insoluble prolamin proteins of sorghum. Caco-2 cell zinc bioaccessibility is reduced with bambara groundnut inclusion. This is due to the increase in phytate content with bambara groundnut inclusion.

In-barrel extrusion moisture has no significant effect on extrudate Caco-2 cell mineral bioaccessibility. However, extrusion cooking at high extrusion moisture improves the protein digestibility of sorghum-bambara groundnut composite extrudates, compared to low extrusion moisture. The more severe nature of low moisture extrusion cooking probably yields less soluble proteins for digestion and a greater decrease in available amino acids due to the Maillard reaction. Research is required to determine the effect of bambara groundnut inclusion and different extrusion moistures on essential amino acid availability.

Extrudate expansion ratio decreases with bambara groundnut inclusion. The dilution of starch with bambara groundnut inclusion probably limits the ability of the extrudate melt to expand and for extrudates to retain an expanded, porous structure. High extrusion moisture also causes less expanded extrudates. Low melt viscosity at high extrusion moisture inhibits the extrudate melt to stretch into an expanded structure due to loss of viscoelasticity of the extrusion melt.

The inclusion of bambara groundnuts with sorghum lowers peak, trough and final paste viscosities of the extrudate flours when heated and in water. This is probably due to reduced starch content. Low moisture extrusion cooking also yields lower paste viscosities compared to high extrusion moisture. The severity of low moisture extrusion probably yields more dextrinized starch.

Extrudate flours from all treatments could have application as instant porridges because of the high initial paste viscosities and WAI. Sorghum-bambara groundnut composite flour extruded at high in-barrel moisture can produce an extrudate flour for porridge making that has excellent protein digestibility. Composite flour extruded at low in-barrel moisture produces an extrudate flour with slightly lower protein digestibility than at high moisture extrusion. Low moisture extrusion of composite flour, however, would enable more nutrient-dense porridge to be produced due to its lower paste viscosity. Bambara groundnut flour alone, when extruded at high in-barrel moisture, can also be applied as a protein-rich nutritious paste with excellent nutrient-density. Sorghum-bambara groundnut composite flour extruded at low in-barrel moisture produce a highly expanded extrudate which could be used as a nutritious expanded snack.

This research established that products such as expanded snacks or instant porridges from sorghum-bambara groundnut composite extrudates could address Protein-Energy Malnutrition (PEM) through improved protein intake and digestibility. A serving size of 50 g (db) extruded composite could contribute approximately 81% of the daily protein requirements of children aged 2-5 years. Furthermore, these products could also improve the children's mineral intake.

It is proposed that the nutritional, physical and functional properties of the extrudates should be compared to commercial similar products such as a maize-soy instant porridge or maize-based expanded snacks. Descriptive sensory analysis should be performed to characterise extrudates and to establish how extrusion moisture levels and bambara groundnut inclusion affect the sensory properties of extrudates. Additionally, the application of more levels of extrusion moisture and bambara groundnut inclusion is proposed to enable the application of response surface methodology to predict and enable the manufacture of extrudates with optimised nutritional, physical, functional and sensory attributes. Costing of the products is necessary to establish the viability of sorghum-bambara groundnut extruded foods in poor communities where PEM is prevalent.

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8 APPENDIX

8.1 Presented from research

K. Muller, Dr J. Kruger, Prof. J.R.N. Taylor and Prof. A. Minnaar, Utilisation of Sorghum and Bambara groundnuts for the Production of Nutritious and Ready-to-eat Foods using Twin Screw Extrusion Cooking. Poster presentation at the SAAFoST Biennial International Congress and Exhibition, 2015. Winner of the Ginsburg Award for the best poster presented by a SAAFoST Young Scientist.

UTILISATION OF SORGHUM AND BAMBARA GROUNDNUTS FOR THE PRODUCTION OF NUTRITIOUS AND READY-TO-EAT FOODS USING TWIN SCREW EXTRUSION COOKING

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INTRODUCTION

Undernourishment can cause Protein Energy Malnutrition (PEM) which is prevalent in many African countries[1].

This is often due to a monotonous cereal-based diet.

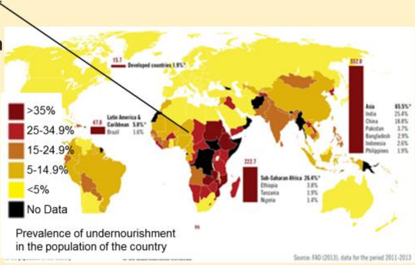


Figure 1 The world map of undernourishment 2011-2013 [2]

Compositing diets with drought resistant and underutilised indigenous legumes can improve the protein quality of starchy diets[3]. Extrusion cooking can aid in the utilisation of legumes such as bambara groundnuts together with starchy cereals, for example sorghum, to produce nutritious, ready-to-eat products. Different extrusion cooking conditions can yield products with various functional and nutritional properties.

OBJECTIVE

To determine the effects of extruding decorticated sorghum flour and whole grain bambara groundnut flour, on their own, and as 50:50 composite under different in-barrel moisture conditions (24 and 40%) on the functional properties and protein quality of final extrudates.

EXPERIMENTAL DESIGN

Decorticated sorghum

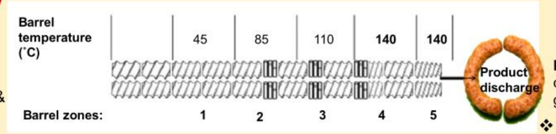
+

Whole grain bambara groundnuts

Hammer milling x 2 (sieve size: 1 mm)

Compositing flours
Sorghum: Bambara groundnuts 50:50

Twin screw Extrusion cooking
In-barrel moisture: **24% (low)** & **40% (high)**
Feed rate: 25 kg/h



Drying & milling

Analyses

- Water absorbance index (WAI)
- Water solubility index (WSI)
- Nitrogen solubility index (NSI)
- Expansion ratio (ER)
- Protein content

RESULTS and DISCUSSION

Table The effects of extrusion cooking at different in-barrel moisture contents and compositing on the protein content, water absorbance index and expansion ratio of dehulled sorghum and whole grain bambara groundnut flours

Flour composition	Extrusion cooking treatment	Protein content (g/100 g)[1]	Water Absorbance Index (g/g)[1]	Expansion Ratio ($d_{extrudate}/d_{die}$)[1]
(sorghum)	**None**	11.3 ± 0.4	2.7 ± 0.1	NA[2]
	24 % moisture	13.4 ± 0.2	6.6 ± 0.1	3.0 ± 0.1
	40 % moisture	12.8 ± 0.2	6.7 ± 0.2	2.3 ± 0.1
(bambara groundnut)	**None**	22.9 ± 0.3	2.3 ± 0.3	NA
	24 % moisture	21.9 ± 0.3	4.6 ± 0.5	2.6 ± 0.1
	40 % moisture	24.0 ± 2.5	6.6 ± 0.9	3.0 ± 0.4
(composite)	**None**	17.0 ± 0.1	2.8 ± 0.0	NA
	24 % moisture	19.2 ± 1.1	7.0 ± 0.2	2.7 ± 0.2
	40 % moisture	19.0 ± 1.3	6.8 ± 0.1	2.3 ± 0.1

1 Results presented on **dry basis** displayed as: mean ± SD (n=2 replicates)
2 NA – Not applicable

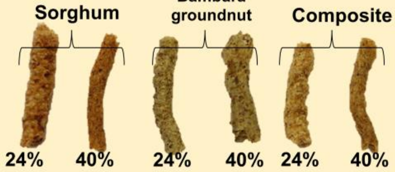


Figure 1 The extrudates of decorticated sorghum and whole grain bambara groundnuts, respectively and composited, extruded at 24 and 40 % in-barrel moisture contents

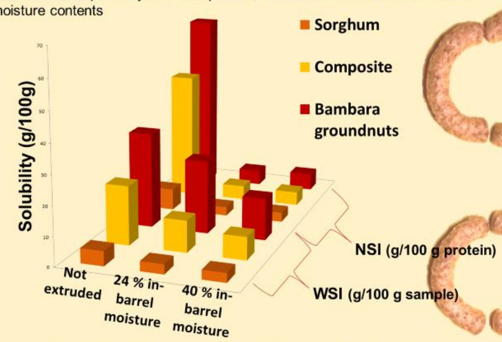


Figure 2 The effects of extrusion cooking at different in-barrel moisture contents and compositing on the WSI and NSI of dehulled sorghum and whole grain bambara groundnut flours

- The WAI of all flours were improved by extrusion cooking (Table).
- Expansion decreased with the addition of bambara groundnuts due to an increase in protein content (Table and Fig. 1). Since ER is dependent on starch gelatinisation, the dilution of starch probably caused the decrease in ER[4].
- WSI and NSI correlated positively (r=0.71) which was also evident in Figure 2. This suggested that the soluble fractions of samples depended greatly on the solubility of proteins present.
- NSI of all flours decreased after extrusion cooking (Fig. 2) presumably due to protein denaturation at hot, moist barrel conditions[5]. The addition of bambara groundnut flour caused increased NSI (Fig. 2) due to a high protein solubility[6].

CONCLUSION

A sorghum-bambara groundnut composite flour extruded at 24 % in-barrel moisture conditions yields a potential expanded snack with superior protein content to 100% sorghum flour. In milled form, this product can potentially be used as an instantised porridge with excellent functionality due to increased WAI.

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