# The processing characteristics of Nigerian pigeon pea (*Cajanus cajan L.*)

By

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# Abstract

This study sorts to improve the utilisation of pigeon pea as a ready-to-eat expanded snack food by investigating it's processing characteristics under different environments. Existing literature suggests that pigeon peas, as reported for other legumes, could be difficult to cook due to their lack of hydration and the time required for softening during heating. These difficulties were attributed to the presence of seed coat, cell wall materials and some anti-nutritional factors. During these studies, the physicochemical characteristics of three commercial varieties of Nigerian pigeon pea (white Agatu, red Agatu and Bayelsa) were determined to select a variety appropriate for development of a snack. A high specific density (1.26 g/ml) of the seed suggested that the seeds' native matrix and components are densely packed with limited voids between structures. Among the findings of this study include contrary to the earlier reports, that the hard to cook textural defect of pigeon pea was equally because of the presence of the seed coat and cell wall, the seed coat did not show to have posed any difficulty in the hydration of the seed. The seed coat easily detached from the cotyledon within 30 minutes of soaking in water after taking its own weight in water. However, the cotyledon weight only increased by 29%. Although soaking and dehulling altered the chemical composition of the seed but the protein, starch and fibre content still showed appropriate values for the production of healthy snacks. As no significant differences ($P<0.05$) in the key physicochemical characteristics were observed for the different varieties and due to economic considerations, white Agatu was selected for further study. To increase accessibility of starch to water, several treatments were applied, either targeting hydration or softening of the seed. In contrast to conventional soaking, hydration without stirring at 37-90 °C for 4 hours revealed three stages of hydration with water uptake levels being similar, but reaching the maximum hydration at different times (120-150 min) depending on the temperature. Cell wall degradation was affected by mild shear and heat, with or without alkaline solutions and enzymes that degrade cell walls. Results showed an improved swelling in excess water of treated milled material suggesting the positive influence of such treatments. For most treated samples, the thermal and crystallinity properties evaluated with Differential Scanning Calorimeter (DSC) and Wide-Angle X-Ray Diffractometer confirmed intact starch crystalline structures. This indicated the robustness and thermostability of pigeon pea. Since the treatments did not cause sufficient change in the hydrated seeds, a severe treatment (thermomechanical extrusion) using high shear and temperatures was used. The twin screw thermomechanical extrusion was adopted to ensure changes in the native chemical components and cellular structures of pigeon pea. Extrusion of treated and non-treated pigeon pea allowed expanded ready-to-eat snack products to be developed that were influenced by extrusion conditions and feed type. Optimised extrusion conditions (11% feed moisture, 14 kg feed rate,

150 °C die temperature and screw speed of 400 rpm) provided maximum torque and caused high levels of starch conversion and positive extrudate quality. Although this has not been reported in literature, the intensity of an X-Ray diffraction peak at $34^0$ 2-Theta (proposed to be globoid crystals of phytic acid salt; an anti-nutritional factor) which was detected in all the seed powdered samples was reduced after the extrusion process. From microscopy and other analyses, it was inferred that for an expanded snack, most of the structural entities that occurred in the seed needed to be eradicated and a homogenous melt favoured formation of well expanded and stable extrudates. Given the above processes applied, it is recommended that white Agatu pigeon pea be used to create extruded snack product in a safe and economic way.
**Keywords**: pigeon pea, cell wall, starch, hydration, swelling, structural degradation and extrusion.

Give thanks to God for He is good, and His love endures forever.
(Ps 118:1)

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# 1 Introduction

## 1.1 Background of the study

In a world of limited resources, the optimum use of materials of high nutritional benefits is essential. Pigeon pea is a drought tolerant legume seed with a rich nutritional composition and can be widely grown in countries where natural resources need to be used prudently. Similar to other legumes, the difficulties in hydration and softening of pigeon pea seeds during cooking may limit the use of this material. This phenomenon is generally referred to as the "hard to cook defect"(Aguilera and Stanley, 1985, Garcia-Vela and Stanley, 1989, Garcia and Lajolo, 1994) and needs to be overcome if the plant seeds are to be safe to eat and provide the nutritional benefits that could be available.

The prolonged time required in the hydration and cooking of pigeon pea seed has been associated with the non-permeability of the seed coat and cell wall materials(Plhak et al., 1987, Aguilera and Rivera, 1992). However, the dense and compact matrix of the native seed structure (the starch and protein components of the seed are all embedded into a strong matrix together with the cell wall materials of the cotyledon) could limit the effectiveness of hydration and subsequent processing. Cooking would normally be associated with the loss of starch native structures and as the gelatinisation of starch (starch conversion) is influenced by moisture, temperature and shear (Hoover and Manuel, 1996, Wang and Copeland, 2012), these are critical factors that influence starch functionality during processing and subsequent digestion. Legume starches, with their C type crystallinity, have been characterised as being very stable in comparison to other starches. Their restricted swelling, poor granule dispensability (disintegration in water) and high temperature gelatinisation transitions contributes to the requirement for high energy consumption during processing (Hoover and Ratnayake, 2002). The limited changes to the starch on heating may also mean that the starches are physically entrapped

by the cell wall matrix and therefore would be more resistant to enzymatic hydrolysis (Englyst and Hudson, 1996).

There are food safety aspects associated with the hard to cook phenomenon in that several anti-nutritional factors may be present in legumes and these may be reduced through soaking and processing. However, long term soaking at warm-ambient conditions may allow excessive microbial contamination and growth. A process that could comminute and cook the pigeon pea and create a different type of product may be an excellent way of encouraging the safe consumption of this underutilised crop for human consumption. Today many starch based materials are used in thermomechanical extrusion to form ready to eat snacks. The high temperatures and shear within a twin screw extruder could process the pigeon pea sufficiently for a snack product to be made. Considering the short residence time of material in extrusion cooking and the importance of starch gelatinisation and protein denaturation during food processing and human digestion some pre-conditioning treatment may be required to counter these limitations.

A value-added product based on Pigeon pea could increase the availability of food to a growing world population and would support food security. To produce such a value-added product and to be able to apply learnings from the work, the transition of pigeon pea during all the relevant processing operations needs to be understood. The work described in this thesis is therefore expected to provide information to both producers and consumers on the characteristics of pigeon pea under preliminary processing regimes that would improve the hydration, swelling and softening of the seed prior to processing into stable products. The thermomechanical extrusion of the pigeon pea as an example of production of a ready to eat snack was studied and described in terms of a possible processing technology and the changes that occur to the seed materials.

## 1.2 Objectives of the study

The evaluation of the behaviour of pigeon pea seed under different processing regimes and production of edible snacks was the main focus of the PhD study. To understand and investigate the processing of the pigeon pea behaviour, some general research objectives were set. These included:

(1) To review the literature to ensure the understanding of the materials and processes available for the project. Chapter two: Literature review. An initial review of literature relating to the research topic.

(2) To provide information of the appropriate methods to use in the study of pigeon pea and it's processing. Chapter three: Materials and methods.

(3) To make appropriate selection of seeds with good physicochemical properties as it would ensure good quality final products. Chapter four: the evaluation of the physicochemical properties of three varieties of pigeon pea and the selection of one variety for the extrusion cooking.

(4) To optimise the hydration of the seed through different techniques involving hydrothermal, alkaline and enzymatic action. Chapter five: The hydration and softening processes to improve starch access to water.

(5) To monitor the swelling behaviour of the dispersion from milled powder under quiescent and mild shear environment. Chapter 4 and 5.

(6) To assess the thermal and structural properties in order to confirm where changes occurred in the native granule crystalline structure. Chapter 5 and 6.

(7) To use the selected treated material in comparison with non-treated feed to produce an expanded edible snacks through thermo-mechanical extrusion. Chapter six: development of extruded products and Chapter seven: the nutritional qualities of the extrudates.

(8) To use information from the project and the literature to create a general conclusion and define additional work that is necessary to fulfil future objectives. Chapter 8: General conclusion.

# 2 Literature Review

## 2.1 Pigeon pea an underutilised indigenous crop

### 2.1.1 Agricultural and food security considerations for pigeon pea

Pigeon pea (*Cajanus cajan* L) belongs to the Leguminosae (Fabaceae) family of flowering plants and is a dicot. This is a drought-tolerant legume suitable for the nutrient-depleted soils of Africa (Odeny, 2007). Pigeon pea is usually stored dry after harvest at moisture contents of about 11-13% and it can be grown in areas where it could become a very important source of nutrition and provide food security benefits. Currently it is mainly eaten in developing countries as an alternative source of protein to the high cost animal proteins (Joseph et al., 1993, Oboh et al., 2000, Belitz et al., 2009). In Nigeria, pigeon pea is grown extensively in the areas of Anambra, Benue and Enugu states. Among the Nigerian languages, it is called *fio-fio/Agbugbu* in Igbo, *Waken Kurawa* in Hausa or *Otili* in Yoruba ((Enwere et al., 1998). Whereas in India, where the pigeon pea is originated from, it is commonly called *red gram* or *Dhals* after dehulling and splitting of the seed (Mahadevamma et al., 2003, Saxena et al., 2010, Vasishtha et al., 2012). Traditionally, the dry pigeon pea seeds are soaked for 12 hours for re-hydration prior to cooking. This extended preparation of the seeds is due to a phenomenon known as the "hard to cook defect" of the seed. The hard to cook defect results in the need for extended soaking periods because of poor water uptake by the dried seeds and prolonged cooking times. The seeds are typically cooked for 2-3 hour before they soften and are ready for consumption (Oboh et al., 2000). Pigeon pea can be eaten whole as vegetable or mixed with coked yam, maize, dried cocoyam grits (*Achicha*) or sweet potato (Enwere, 1998). Although this crop is used widely in its natural forms, its industrial application is limited due to the hard texture (hard to cook) of the seed and unavailable information on the material properties during processing.

### 2.1.2 Nutritional aspect of pigeon pea seed components

The chemical composition of the matured pigeon pea seed (Figure 2-1) on wet basis consists of protein: 19-24%, starch: 45- 53%, fat: 2.4-1.9%, crude fibre: 6.6% and soluble sugar: 3.1-6.0% (Saxena et al., 2010)

Until 30 years ago, pigeon pea was only consumed in the conventional form as a dietary source of protein in developing nations. However, recently investigations have shown that other macro (starch and dietary fibre) and micro nutrient (minerals and phytochemical) associated with the composition of pigeon pea are highly beneficial to man (Singh, 1990, Rampersad et al 2003, Saxena et al., 2010, Oke, 2014) and thus its use may be extended. Within the 68% carbohydrate content occurring in the pigeon pea, around 40% will be starch, but importantly it is thought that 60% of the starch will be resistant starch that defers digestion. While 24% of the carbohydrate may be dietary fibre, of which 73% are non-starch polysaccharides (cell wall materials) while the remaining is in the form of lignin (Vasishtha et al., 2012). Due to the high content of resistant starch and dietary fibre, consumption of pigeon pea results in a low (24) glycaemic index (Apata, 2008, Kaur and Sandhu, 2010b, Oboh et al., 2010). Similar to other legumes, the dietary fibres components can be expected to contribute to the reduction and prevention of digestive and coronary diseases (Waldron et al., 2003, Albersheim et al., 2011, Vasishtha et al., 2012, Arnoldi et al., 2015). Reports show that pigeon pea is rated low in saturated fat, cholesterol and sodium (Mayzelle and Bell, 2014), while calcium (120.8 mg/g) and magnesium (122mg/g) are present in high proportions (Saxena et al., 2010).

### 2.1.3 Pigeon pea seed matrix structure

The components of the pigeon pea seeds are typical of the seeds of other legumes. The seeds are made up of three distinct components: namely, seed coat (testa): 14% (by weight), embryo: 1% and cotyledon: 85%

(Figure 2-2). The seed coat, comprising of the secondary cell walls is the outer tissue covering the cotyledon cells. It controls the imbibition and absorption of water into the seed. The basic cell layers of the seed coat from the outer surface to the layer attached to the cotyledon are the waxy cuticle, epidermis (palisade), hypodermis and the interior parenchyma (Souza and Marcos-Filho, 2001, MoÏSe et al., 2005). The seed coat is mostly formed with cell wall materials (cellulose, hemicellulose, lignin and pectin) but also contains flavonoids, proteins, peptides, amino acids, alkaloids, terpenoids and steroids. The seed coat cell wall materials, together with the cotyledon cell wall materials are often associated with the regulation of water uptake into the seed.

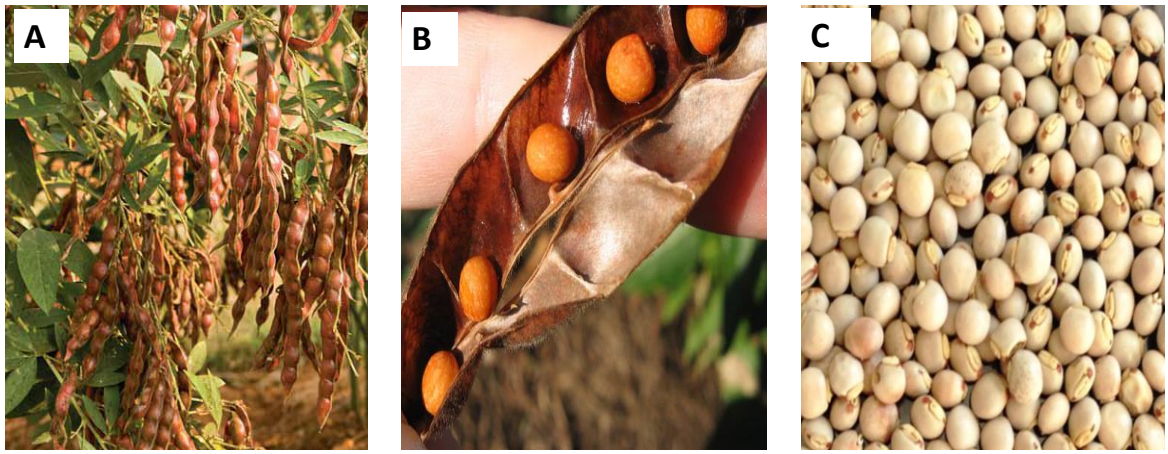



**Figure 2-1**Mature pigeon pea seed (A) intact pigeon pea pod (B) opened pigeon pea pod (C) Dry and mature pigeon pea seeds.
Sources: (A) ICRISAT 2012 (B) transitionfrasercoast.org.au,
(C) Unpublished image from laboratory.

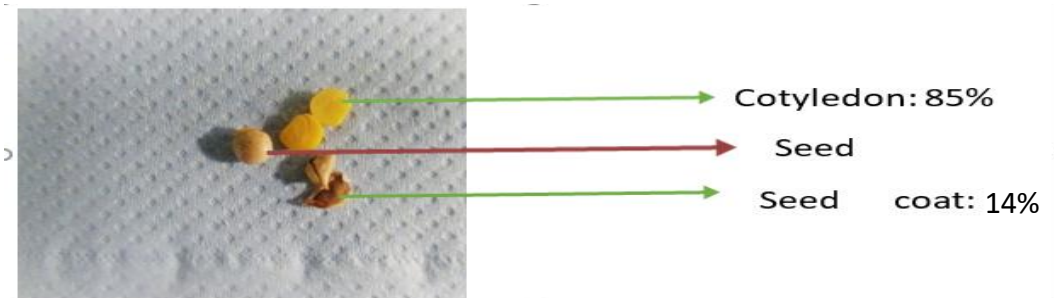
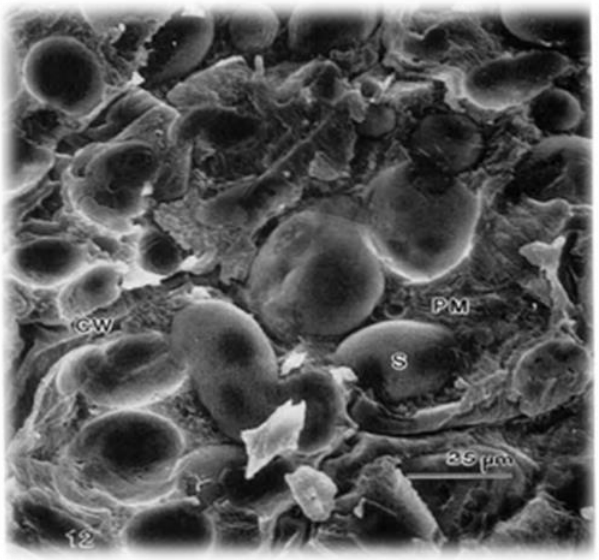



**Figure 2-2 :** Pigeon pea seed components.




PM: protein matrix
CW: cell wall
S: starch granule

**Figure 2-3:** Microstructure of pigeon pea cotyledon cell.
Source: (Joseph et al., 1993).

The cells associated with the cotyledon Figure 2-3 are differentiated by the cell walls (CW). Within the cells are starch granules (S) that are about 27 x 43 µm in size and are embedded within a protein matrix (PM) that also contains protein bodies (Joseph et al., 1993) which are still surrounded by membranes containing lipid bodies, as found in pea cotyledon cells (Aykroyd et al., 1982). The cotyledon contains about 60-90% of the total seed cell wall materials (Vasishtha et al., 2012) with the majority (40%) of the cotyledon cell wall material consisting of pectic polysaccharides.

## 2.2 Limitations on current utilisation

Despite the health benefits, many factors have limited processing of pigeon pea at domestic and industrial level. For it to be safe to eat and acceptable as a nutritious and quality product, it requires extensive heat treatment. This is because like other legumes, the pigeon peas seed is hard to hydrate. Hydration is essential for food applications and to negate the potential of the anti-nutritional factors that occur in this seed and to increase the digestibility of nutrients. If the seed is not adequately hydrated, then extensive thermal processing is required, and this uses extensive energy and can cause a negative impact on the material that is hydrated. The

major chemical components of pigeon pea; starch, non-starch polysaccharides and protein need hydration for adequate cooking before consumption.

Among the limiting factors that need to be overcome during cooking is the "hard to cook defect". Also, the presence of some anti-nutritional factors (phytates, oligosaccharides, polyphenol, lectins and oxalate) impair consumption and processing of pigeon pea (Onwuka, 2006).

### 2.2.1 Hard to cook defect (HTC) of seed

The hard to cook defect (HTC) is a textural defect developed during post-harvest storage (and exhibited by dry legume seed during cooking in which the seeds are hard to hydrate and cotyledon cells fail to separate. This defect could lead to a delay in starch gelatinisation, protein denaturation, softening of seed, an increase in cooking time and seed hardness when compared to the fresh seed. Hence, domestic and commercial consumers have a low acceptability of HTC seeds (Stanley and Aguilera, 1985, Plhak et al., 1987, Garcia-Vela and Stanley, 1989, Shomer et al., 1990, Martín-Cabrejas et al., 1997).

Several investigations have shown that the hard to cook defect (HTC) is developed during the initial 2 months of storage of harvested dry seed when they are stored at high humidity (above 65%) and at temperatures above 37 °C, as will occur in the tropics (Del Valle et al., 1992a, Shiga et al., 2009). The major mechanisms, which are thought to contribute to the development of HTC in seeds, include interactions within the cell walls. Failure of phytic acid in the protein bodies of the cotyledon to chelate $Ca^{2+}$ and $Mg^{2+}$ and possible hydrolysis of phytate by phytase to release $Ca^{2+}$ and $Mg^{2+}$, allows the divalent ions to retain their cross-linking action within the pectin and this maintains a robust structure within the middle lamella of the cell walls. Due to the decrease in esterification of pectin, the crosslinking results in the formation of insoluble pectate in the middle lamella. Other mechanism suggested for the formation of HTC defect

includes polymerisation of seed coat cell wall polyphenols with protein from cotyledon protein bodies leading to lignification of the middle lamella. Interactions of cell wall polymers and limited intra and intercellular water availability also contributes to this textural defect (Garcia-Vela and Stanley, 1989, Hentges et al., 1991, Del Valle and Stanley, 1995, Waldron et al., 2003, Shiga et al., 2009). Hentges et al. (1991) proposed that starch granule and protein bodies might be involved in HTC defect due to the low solubility of amylose and protein of HTC seeds.

#### 2.2.1.1 Impact of HTC on the seed structures and properties

HTC influences some undesirable effects for the consumption of HTC seeds due to the failure of the seed to become tender during cooking resulting in prolonged cooking. Its commercial applications and values are therefore limited (Yousif et al., 2002) and certain pre-treatments which increases cellular hydration and decreases heating are required (Kigel, 1999).
The HTC defect can be reversed (softening of the hard seed) if it occurs due to the pectin-phytate mechanism, but would be irreversible if occurring due to lignification. The water activity of the stored seed influences the reversible mechanism, while the storage at high temperature increases the irreversible HTC defect (Del Valle and Stanley, 1995).

In an assessment of HTC on the migration of water into the seeds, it was observed that the movement of water during hydration is different in HTC and non-HTC seeds. Higher loss of water occurs during dehydration of soaked HTC seeds than in non-HTC seeds, even though they had similar initial moisture contents. It was suggested that the water absorbed bound differently in the microstructure of HTC and non-HTC seeds. Although, Del Valle et al., (1992b), reported that the rate of water uptake by HTC seeds is reduced compared to non-HTC seeds, yet the rate of dehydration of HTC seed was faster. It was considered that most of water absorbed during hydration was free water. Faster drying rates was observed at the beginning of drying of HTC samples, when the temperatures were lower

than 75 °C, due to the dehydrating water acting as free water and its loss being controlled by mass and heat transfer. At higher temperatures (above 75 °C) in soaked non-HTC seed, the drying rate was slower as most of the imbibed water present in the cotyledon cells was associated with gelatinised starch. Hence, the diffusion of water vapour during drying from the cell of the non-HTC sample would be difficult compared to HTC seed where the initial water absorbed were held in the interstitial spaces between seed coat and cotyledon (Hincks and Stanley, 1986, Aguilera and Rivera, 1992, Yousif et al., 2002).

The hardness of the seeds is used in evaluating the impact of cooking or pre-treatment in the reduction of HTC. Generally the hardness is measured using texture analysis and the peak deformation force is often related to the degree of softening of the seed (Kigel, 1999). Since the middle lamella of HTC seeds fails to soften and dissolve during cooking there is an absence of cell separation and this impacts on the perceived softness of the seed. The adherence of the middle lamella to the intercellular cells and intercellular spaces affects the cell separation (Liu et al., 1993a).

Another approach in the understanding of the hard to cook defect is to monitor the starch gelatinisation behaviour since cooking often implies loss of starch granule structure (conversion from a semi-crystalline granule to an amorphous material). Evidence showing limited starch gelatinisation in HTC seed can be the retention of birefringence of starch granules at the core of HTC seeds. The limited accessibility of HTC cotyledon cells to water could stop starch gelatinisation and reduce the pressures within cells which would be associated with starch granule swelling and rupture. Evidence has shown that about 50% of HTC starch are non-gelatinised during short time cooking (Hohlberg and Stanley, 1987, Liu et al., 1992, Garcia and Lajolo, 1994).

Heat treatment can lead to association and dissociation of protein and may even cause formation of gel networks. Dissociation of the proteins' constituent subunits may occur and there may be unfolding of the protein

structures causing surface exposure of the hydrophobic groups within the protein. The complexity exhibited during association and dissociation reactions of protein and the interaction between the embedded starch and the protein matrix could affect the solubility of protein and also change the behaviour of the starch(Stanley and Aguilera, 1985, Ralet et al., 1993, Noah et al., 1998, Martín-Cabrejas et al., 1999, Aguilera et al., 2000).

### 2.2.2 Anti-nutritional factors (ANFs)

Anti-nutritional factors (ANFs) are secondary metabolites that are naturally inherent components of plants, but which interfere with human nutrition, especially in digestion and utilization of nutrients. AFNs are low molecular weight compounds synthesized by the plant in order to have a defence against predatory organisms (Wink, 2010). These secondary metabolites have affected the human and animal consumption of legume foods, despite the abundant availability of nutrients (carbohydrates, protein, vitamins and trace minerals) found in them. The quality of food depends only on the availability of nutrient after ingestion. ANFs may affect health by being eaten, but also some have an impact if inhaled or absorbed through the skin (Alonso et al., 2000b).

The presence of some anti-nutritional factors have been detected in pigeon pea and can be classified as heat labile (e.g. protein inhibitors and lectin) or heat stable (e.g. phytate, tannin or proanthocyandins, oligosaccharides, saponins, oxalate and alkaloids) (Onwuka, 2006, Soetan and Oyewole, 2009). All these secondary metabolites interfere with nutrient availability, except oligosaccharides that are indigestible due to absence of α galactosidase in the humans' digestive tract and saponins that impact a bitter taste.

It is necessary to review the toxicity of pigeon pea ANFs in human system since their presence affects the utilization of pigeon pea as food crop (Onimawo and Akpojovwo, 2006). The choice of selected anti nutritional

factors mentioned in the following section was based on the severity of the health effect on human exposure even if it is on long term exposure to relatively low levels.

ANFs levels are typically higher in raw seeds than in the processed materials. The natural dose of ANF in food does not indicate their safety as adverse health effect results upon acute and chronic exposure. Food is only regarded as safe when consumed at recommended level. Several processing techniques such as soaking, cooking, germination and fermentation have been applied in an attempt to reduce the level and negative impact of anti- nutrients. Recently, for better improvement of nutritional yield, certain enzymes have been successfully employed by several authors and industries as a way of reducing the anti–nutritional content of legume flour (Soetan and Oyewole, 2009).

#### 2.2.2.1 Alkaloids

Alkaloids are naturally occurring toxic plant secondary metabolites and are heterocyclic nitrogenous compound. Many amino acids act as precursors. The toxicity pathway for the alkaloids depends on the type of alkaloid. Among the toxic alkaloids are quinolizidines and this is found in lupins, which is a member of Fabaceae family of which pigeon pea belongs (Prakash et al., 1999).

#### 2.2.2.2 Cyanide

Cyanogenic glycosides are compartmentalised in the vacuoles of plant cells and hence are dissociated from their hydrolytic enzymes. If the compartment is destroyed, by the disruption of cells during food processing or maceration of ingested food, it will initiate the hydrolysis of cyanogenic glycoside by glucosidases. This then leads to formation of hydrogen cyanide (HCN), a new metabolite that is highly toxic. The volatile nature of hydrogen cyanide makes it easier for liberation through the plant tissue into human cells by inhalation, skin and eye contact Sensitivity to cyanide toxicity depends on age, body mass and health status of individual. Chronic

exposure causes malnutrition, diabetes, congenital malfunctions, and neurological disorder. Strict observance of safe limit dose between 0.3-0.5mg/kg body weight is highly recommended. In vitro toxicity could be reduced through inactivation of glycosidase by high temperature and vaporisation of HCN. About 40mg/100kg of HCN has been reported on some variety of pigeon pea (Rietjens et al., 2005, Onwuka, 2006).

#### 2.2.2.3 Lectin (Hemagglutinin)

Lectins are sugar binding proteins known for their binding specificity and precipitation of sugar moieties (glycoprotein and polysaccharides). They bind specific sugar resulting in agglutination of the blood cells. The lectins remain biological active during their passage through the gastrointestinal tract resisting both degradation by proteolytic enzymes and micro flora of the gut. The majority of lectins are destroyed by heat (Onwuka, 2006), but some like ricin (castor oil beans) are not very susceptible to heat treatment.

#### 2.2.2.4 Oxalate

Oxalic acid forms water soluble salts with $Na^+$, $K^+$, and $NH4^+$ ions. It also binds with $Ca^{2+}$, $Fe^{2+}$ and $Mg^{2+}$ rendering these minerals unavailable for human nutrition. Oxalate may be removed from the food by leaching in water during soaking and germination. Cooking after soaking or germination reduces oxalate, while dehulling decreases it by 38% blanching and fermentation are also possible ways for reduction(Gimba et al., 2013).

#### 2.2.2.5 Phytic acid

Phytic acid (PA) chemical known as myoinositol (1, 2, 3, 4, 5, 6) hexakisphosphoric acid, is the primary storage compound of phosphorous in seeds. The phytic acid occurs in the protein storage vacuoles as globoids crystal structure in complex with minerals where it may account for 80% of the total seed phosphorous. The negatively charged phosphate in PA binds (chelate) with metallic cations of Ca, Fe, K, Mg, Mn and Zn to form an insoluble solid called phytate, thereby making the minerals insoluble and

unavailable for human nutrition (Maenz,D.D., 2001, Lopez et al., 2002, Bohn et al., 2008). Phytate forms a strong complex with some proteins and resists their proteolysis at pH lower than the isoelectric pH of protein (Kumar et al., 2010). Ways of elimination or reduction of PA include milling to remove the bran (seed coat) and soaking which removes up to two thirds of PA by the action of endogenous phytase activity. Heat treatment has minor effects, while germination for 48 h reduces it by 40% (Ologhobo and Fetuga, 1984).

## 2.3 Improving application by hydration and softening of seed

The hydration of the dry seed is an important operation carried out during most food processes to allow migration of water into the dry seed prior to cooking. Usually after harvest, pigeon pea seeds are stored and then marketed as dry seed with a moisture content ranging from 10-14%. The hydration of seed is aimed at increasing the moisture content prior to processing or to soften the seed prior to cooking. The variables that will influence hydration can include temperature, seed moisture content, hydration medium and time. The enhancement of hydration and softening of seed often involve the use of different salt medium (acid, alkali, neutral), cell wall degrading enzyme and combination of temperature higher or lower than starch gelatinisation temperature with pressure or vacuum. Usually soaking at ambient conditions (20-37 °C) are more common than at higher temperatures.

Physical or structural changes may occur following the hydration of seed at different conditions. Parameters such as water uptake holding capacity, chemical composition, mechanical, rheological, and thermal properties and microstructure are used in assessing the impact of the hydration. Some seed properties and structural components (such as colour, porosity and thickness of seed coat, waxy cuticle, cell walls, middle lamella, starch granule and cell membranes) impair permeability of water into the seed. The thickness of the seed coat is thought to play a major role in limiting

water penetration into the seed. However, authors have also associated the compositional components (protein, carbohydrates, fat, phytate, polyphenol and lignin) with being involved in controlling the uptake and movement of water inside the seed (Hsu et al., 1983, Swanson et al., 1985, Aguilera and Rivera, 1992, Souza and Marcos-Filho, 2001, Ross et al., 2013).

According to Bewley and Black (1994), three factors affect the imbibition of seed. These factors were osmotic potential, matrix components and pressure potential.

**Osmotic potentials:** The dissolved solutes in the seed cells affects the uptake of water. Water diffuses from higher potential to lower potential. The greater the concentration of cell solutes, the lower the water potential.

**The matrix**: The ability of the cellular matrix components (cell walls, starch and protein bodies) to bind with water influences the water uptake.

**The pressure potentials**: The ability of the cell components to swell as water enters the cell and the resistance by the external cell walls to prevent bursting of cells.

### 2.3.1 Moisture migration during hydration of seeds

The imbibition of water by dry seed during hydration occurs in three phases, with adsorption and absorption occurring in the first phase (Ross et al., 2013), the soaking medium forms a wetting front on the seed coat surface (Kikuchi et al., 2006, Ross et al., 2013). Due to the increased interaction of water at the seed surface, water is adsorbed to form a monolayer through Van der Waal or chemical bonding (Ross et al., 2013). With more water binding on the surface of the seed as time increases, diffusion and capillary movement of water are created at the surface of the seed, which causes water to penetrate inside the seed (Hsu et al., 1983, Bewley and Black, 1994, Ross et al., 2013). At this first phase of hydration, if the whole dry seed is exposed to solvent near ambient temperature, the saturated surface concentration would initiate structural transition (softening and

expansion) of the seed coat with possible separation of cell walls (Ross et al., 2013). The osmotic pressure created by the water as it diffuses into the seed interior and wets the cell, result in rapid imbibition. The hydration pattern in Figure 2-4 shows components responsible for water uptake while Figure 2-5 shows hydration pattern exhibited by well and poor hydrating seeds (Vertucci and Leopold, 1987, Aguilera and Rivera, 1992, Ross et al., 2013).

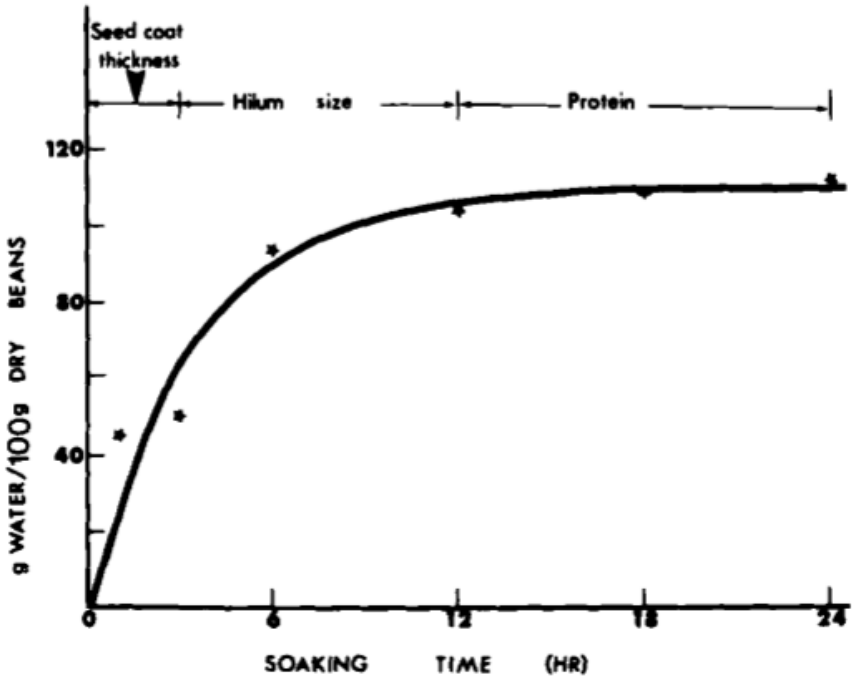



**Figure 2-4:** Generalised water absorption curve showing components accounting for water uptake as a function of time. Source: (Stanley and Aguilera, 1985).

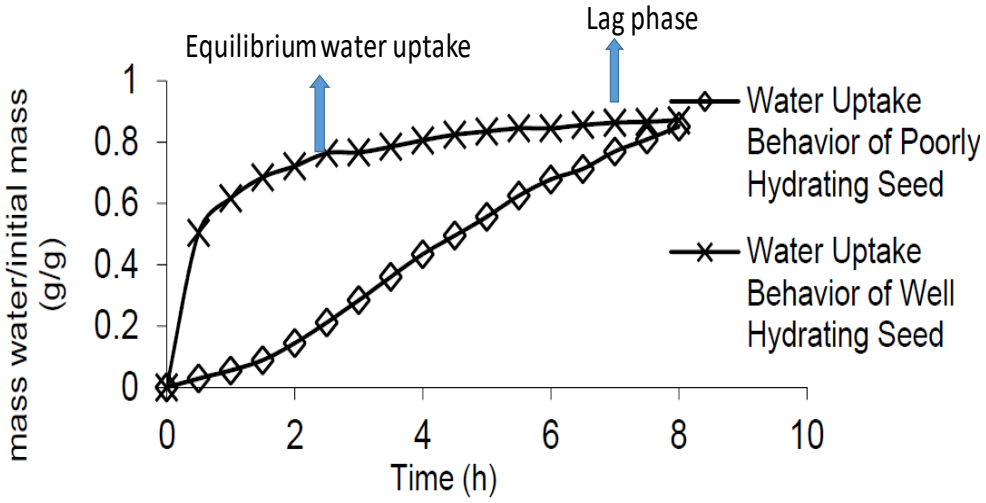



**Figure 2-5:** water uptake of well hydrating and poor hydrating seeds. Source : (Ross et al., 2013).

Del Valle et al, (1992b) described the first phase in seed hydration as initial linear phase, which is followed by a diffusion-controlled stage. Hsu et al., (1983) and Plhak et al., 1989 attributed the initial water uptake in legume seeds to the capillary driven forces required for filling of pores and capillaries at the surface of the seed coat and hilum. The majority of the water imbibed by the seed during hydration is bulk water entrapped in the interstitial spaces (gas spaces) between the seed coat and cotyledon(Plhak et al., 1989). It has been observed that seed coat swells faster than the cotyledon at this initial stage of water uptake (Del Valle et al., 1992c). As the permeability of the seed coat controls the uptake of water, the presence of pigmented seed coat slows the rate of the water due to the greater adhesion of such a coloured seed coat to the cotyledon (Souza and Marcos-Filho, 2001).

In the second phase, a rapid and maximum swelling occurs as water hydrates most of the dry cells and components leading to equilibrium water uptake. This water uptake depends on the size of the seed. The matrix and

pressure potentials influence this phase of imbibition. Due to the weakening of the seed coat cell layers and the difference between the water potentials of the medium and cellular matrix components, water diffuses into the cotyledon (Del Valle et al., 1992c, e Borges et al., 2015). Singh and Kulshrestha (1987) and Ross et al., (2013) described the water uptake into pigeon pea as temperature dependent. It is expected that due to the diffusion action and osmotic potential of the soaking water, that more water would ingress into the cotyledon to modify the cotyledon structural macromolecules thereby resulting in the swelling of the seed(Singh and Kulshrestha, 1987, Vertucci and Leopold, 1987, Del Valle et al., 1992c, Joseph et al., 1993, Joseph et al., 2006, Kikuchi et al., 2006, Shafaei et al., 2014).However, the seed swells according to the ability of the cell membrane to withstand pressure against the cell wall (turgor pressure).

In the third phase, which is the final phase, the hydration rate is slowed down as water uptake enters a lag phase after attaining equilibrium water uptake. At this stage, both the osmotic potentials, the matrix components and the pressure exerted on the external cell wall are halted as the seed attains its full hydration capacity. No more increase in water content of the seed occurs but the leaching of the electrolytes may increase at this stage if the seed is still immersed in soaking medium. Many of the electrolytes are lost from the cell and some from the cell wall (Bewley and Black, 1994). The seed is assumed to have reached the equilibrium moisture content where it neither gains nor loses moisture. Probably, to attain the equilibrium water uptake during soaking, water needs to fill in all the free interstitial spaces in the seed tissue structures. The free interstitial spaces to be filled include spaces between the seed coat and cotyledon, cotyledon protein matrix, protein bodies-starch granules matrix, between starch granules and cellular membrane. According to Vertucci and Leopold (1987), the interstitial water could exist in three forms as bound water to the cotyledon matrix, weakly bound water, or loosely water, which is associated with polymers surface.

Usually water uptake behaviours of the seed are monitored through the plotting of water absorbed against time (Figure 2-4 and Figure 2-5).
A sigmoidal water uptake pattern, which is common to legume seeds (Hsu et al., 1983, Peleg, 1988, Del Valle et al., 1992c) are also exhibited by pigeon pea seed. To indicate the extent of water uptake, the increase in seed size, volume or swelling capacity of hydrated seed may be determined. The water uptake pattern together with other physical observations of the hydrating seed are used to assess the movement of water into the seed and hydration properties of the seed. However, it is expected that the seed coat together with attached structural components (hilum, and micropyle) are pathways through which water penetrates into the seed. More advanced techniques, such as Micro Magnetic Resonance Imaging (MRI) techniques, have been used to confirm migration of water into the seed. Kikuchi et al., (2006) used MRI to confirm that water uptake by leguminous seed (dry bean) is through a specialised tissue (lens) near hilum. Through this lens, the soaking fluid is primarily distributed to the seed coat (testa). Following the entry of water into the seed coat, it moves to the cotyledon through the radicle with an increase in seed size, which corresponds to an increase in water content.

### 2.3.2 Method of enhancing seed hydration and softening

Conventional soaking, higher temperature soaking (hydrothermal treatment), chemical and enzymatic breakdown of the cell wall materials can be applied to improve water uptake and ensure softening of the dry seeds, thereby targeting the reduction or reversibility of the hard to cook defect of the seed.

#### 2.3.2.1 Conventional soaking

Traditionally, soaking at ambient temperature has being a pre–treatment operation for dry legume seeds to increase the moisture content prior to cooking. Conventional soaking involves soaking of the seed at ambient

condition for 12-18 h by immersing the seeds in excess water so that the seed can imbibe water relative to the hydration capacity of the seed. Apart from allowing more water to penetrate into the seed, some soluble anti-nutritional factors are reduced or eliminated during the soaking period (Duhan et al., 1998). Economically, it is reasonable to soak at ambient conditions in order to reduce the cost of processing. Hydration at ambient temperature is best for seed possessing seed coat whose structural transition (softening, expansion deformation) can occur at ambient conditions. However, care would be required to ensure hygienic precautions are taken to prevent any microbial contamination during this process, as the soaking times could be long.

#### 2.3.2.2 High temperature soaking (hydrothermal treatment)

If shorter times were required, an increase in temperature would affect structural changes within the seeds. The impact of the thermal changes will be dependent on the temperature used and the hydration level achieved. Pectin's contribution to the texture is one of the most prominent quality attributes of plant derived food (Waldron et al., 2003, Diaz et al., 2007). Pectin degrades when heated at high temperature and neutral pH to lower molecular weights by the breakage of glycosidic bonds adjacent to carboxymethyl groups. This reaction, known as beta-eliminative degradation, leads to degradation of cell wall protopectin (soluble pectic substances) and middle lamella calcium pectate (insoluble pectic substances) with solubilisation of the middle lamella and reduction of cell-cell adhesion. Therefore cell wall polysaccharides (Saarela, 2011) will be affected if soaking occurs at high temperatures (85-100 °C). The seed texture becomes softer due to cell separation (Liu et al., 1993b). Soluble materials are leached out into the treatment medium due to the degraded cell membrane (Liu et al., 1993b, Waldron et al., 2003, Diaz et al., 2007, Belitz et al., 2009, Saarela, 2011).

As pigeon pea is predominantly a starchy material, soaking will impact on texture by influencing the starch gelatinisation temperature. The increase in water available may be sufficient for starch gelatinisation at the soaking temperature. Seed softening may occur due to the change in state of the starch and the pressure the swelling starch may have on the cellular structures within the cotyledons. Therefore, with appropriate soaking conditions of time, pH and temperature, the loss of cellular integrity and increased porosity could influence seed hydration, cell separation, softening, starch gelatinisation, protein denaturation and swelling.

#### 2.3.2.3 Chemical breakdown of cell wall materials

Originally, during cooking or ripening, seed softening follows a chronological process that involve, dissolution of middle lamella, cell separation, softening of cell wall and finally seed softening. Disruption of the pectate in the middle lamella can occur during cold soaking. Calcium ($Ca^{++}$) and magnesium ($Mg^{++}$) pectate are associated with the hard to cook defect and treatment with salt or chelating agents may reverse this. Chelation and ion exchange of the divalent cations in the pectate with the monovalent cations of the soaking salt solution would cause solubilisation of the pectate (Garcia-Vela et al., 1991, Del Valle et al., 1992a). It was reported that reversal of HTC could occur if seeds were soaked for 18 hours with a chelating agent such as 5% EDTA (ethylenediaminetetraacetic acid) solution, ion exchange solution or with monovalent salts (sodium bicarbonate) solution. This treatment as reported enhanced the disruption of cell wall and cell separation with a two-fold decrease in seed hardness (Garcia-Vela et al., 1991, Aguilera and Rivera, 1992, Del Valle and Stanley, 1995).

While pectin may be solubilised with weak acid or alkali, hemicellulose can be solubilised with alkali. However, due to the chemical stability of its structure (crystalline structure), and extreme insolubility, cellulose is difficult to degrade chemically. It is best to degrade cellulose through

application of exo and endo cell wall degrading enzymes and this is also applicable to lignin components of the cell wall (Albersheim et al., 2011).
Many authors have made observations on the impact of using different chemicals in the soaking of seeds. For example, Aguilera and Rivera (1992) evaluated the effect of soaking black beans in 0.05 M sodium bicarbonate at pH 8 for 18 hours at 20 °C. The study shows that softening of the beans increased with the concentration of salt. The increase in the $Na^+$ content of the soaked beans corresponded to the degree of softening of the seed during cooking. Microstructurally, the treatment disrupted the surface of the cells with the dissolution of the cell wall. The concept put forward was that dissolution of the cell wall improved the hydration of cellular components such as starch and protein. An alternative mechanism was suggested from the data on soaking of seeds in a carbonate solution at pH 10. This treatment resulted in the lowering of the onset denaturation temperature of protein and the increase in seed softening was thought to correspond to the extra protein denaturation rather than pectin solubilisation (Del Valle et al., 1992b).

#### 2.3.2.4 Enzymatic break down of cell wall materials

The enzymatic degradation or biodegradation of cell wall materials (cellulose, hemicellulose, lignin and pectin) involves the hydrolysis of the cell wall materials to higher or lower molecular weight polymers, oligosaccharides and monosaccharides units (de Vries and Visser, 2001). The benefit of this treatment would be to improve the functional properties of the material during processing especially the hydration properties and solution viscosity. The extent of hydrolysis of cell wall materials (substrate) varies according to the enzyme activity and type of substrate involved. This concept of disruption of the cell wall components through commercial cell wall degrading enzymes has been adapted from the natural enzyme degradation of cell wall materials during fruit ripening and microbial spoilage of plant materials.

The commercial cell wall degrading enzymes applied in the cell wall degradation studies are mainly from the genus *Aspergillus,*(a filamentous fungus) though sometimes enzymes from *Bacillus subtilis* are used. The commercial enzymes comprise of either single or mixtures of pectinase, xylanase, cellulase, hemicellulase and beta glucanase. Albersheim et al. (2009) stated that the pectic polysaccharides of dicots (legumes) are preferentially solubilised when treated with cell wall degrading enzymes (polygalacturonase). The application of these enzymes facilitate processing and improve uniform product quality (texture and flavour) through the solubilisation of insoluble fractions (Poutanen, 1997). The application of xylanase in the bakery and milling industry have recorded positive effects in improvement of poor quality flour (Jiménez and Martínez-Anaya, 2001, Courtin and Delcour, 2002). The degradation of arabinoxylan (back bone of hemicellulose) and a major component of cell wall materials changes the bulk product's interactions with moisture (Poutanen, 1997, Courtin and Delcour, 2002, Butt et al., 2008). Using the same concepts, the application of combined cell wall degrading enzymes to pigeon pea seed was expected to enhance hydration, seed softening and final product quality.

## 2.4 Starch and their changes when cooked

The different transitions within starch granules during cooking influence the application and utilisation of starchy foods. The different levels of organisation in the native starch granule and how these may change need to be understood during processing if an optimum product quality is to be achieved. Water and temperature have the greatest impact on the starch, while other processing conditions may also be manipulated to influence the desired transitions.

### 2.4.1 Understanding starch granule structure and organisation

Pigeon pea contains about 44.67- 53.0% starch, which contributes to its great energy potential. Starch is a carbohydrate (polysaccharide) that can be stored as granules in plant material. The starch granule may consist of

two different molecules, which are amylose and amylopectin. Though these molecules have glucose as their building blocks, amylose exists mostly in a linear form with alpha-1,4 linkages (Figure 2-6), while amylopectin is highly branched (Figure 2-6), with alpha-1,4 linked backbone, alpha-1,6 linked branch points and short alpha-1,4 branches. The organisations and interactions of these molecules results in the different levels of structure found in the granule (Zobel, 1988a, Parker and Ring, 2001). During processing, these levels of organisation are disrupted giving rise to the characteristics eating qualities of starchy foods.

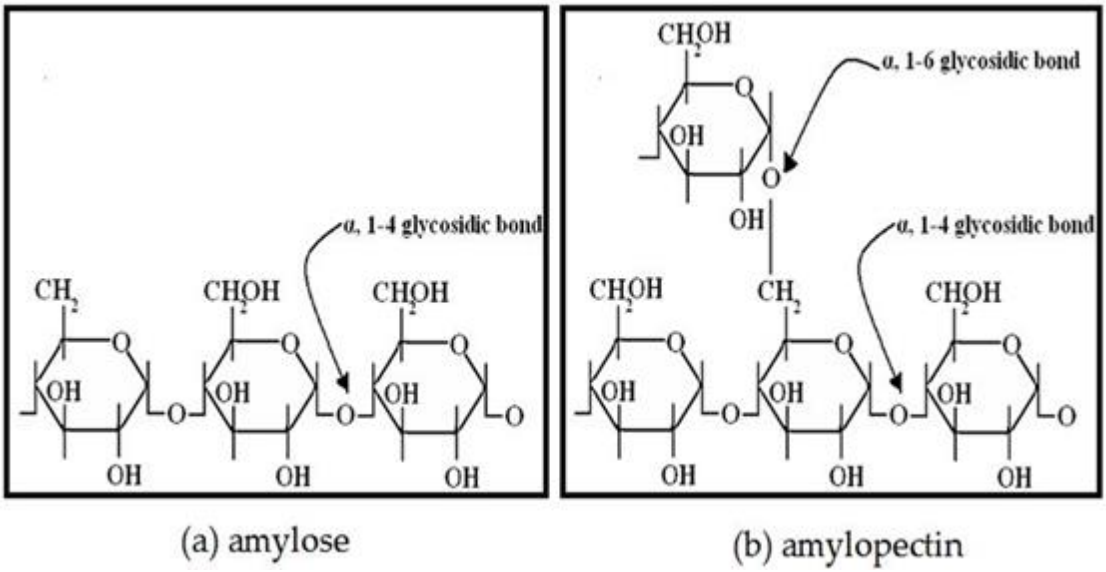



**Figure 2-6:** Schematic diagram of starch two major molecular components; amylose and amylopectin. Source: Caliskan, et al., (2017).

In addition to the formation of starch macromolecules from the basic glucose units, six major levels of starch organisation are discussed in the literature (Figure 2-7). First in the level is the molecular interactions of amylopectin chains into helical structures followed by the second level (structure size: 0.1-1.0 nm) which is the clustering of the different helical chains of amylopectin to form a semi crystalline lamella. Between these lamellae there is an amorphous interspacing region where the helices were not arranged in orderly manner and most of the amylose may reside (Buléon et al., 1998).

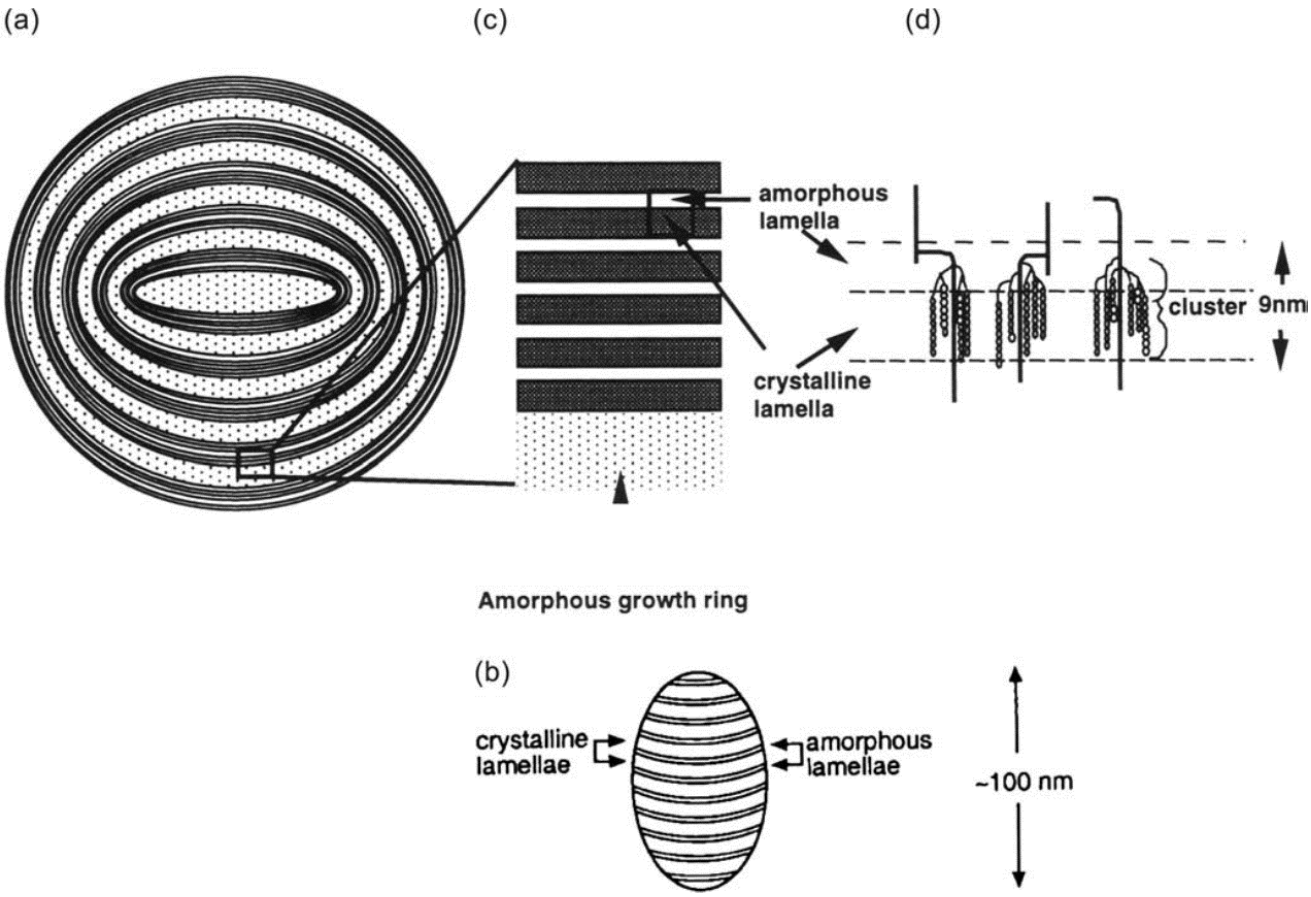



**Figure 2-7:** organisation in starch granule*(a)* amorphous and semi-crystalline growth rings in a starch granule, (b) blocklet, (c) amorphous and crystalline lamellae in a stack and part of an amorphous growth ring, (d) a cluster model of the aligned double helices (from amylopectin side chains) within a crystalline lamella and amylopectin branch points within an amorphous lamella. Source: Vandeputte and Delcour 2004.

Thirdly, together these clusters of double helical starch crystallite lamella and amorphous lamella form regular repeat structures of approximately 9 nm. The helical cluster arrangement gives rise to different types of starch crystallite (polymorphs). For the A form of crystallite, the double helices are arranged in a monoclinic array with limited water (eight water molecules) in the unit cell of the crystallite. Cereal starches are typical of A crystallites, while in B crystallite arrangements, the double helices are packed into hexagonal arrays with thirty-six water molecules in the unit cell of the crystallite. Mostly root and tuber starches are formed as B type.

Within the granules a combination of A and B crystallite forms can exist, and this is typical for legumes. Pigeon pea has been reported to have C type crystallinity which comprises of A and B polymorphs. The A crystallites are found in the peripheral regions of the starch granule while the B crystallites are in the interior (Ball et al., 1996, Gallant et al., 1997, Bogracheva et al., 1998, Buléon et al., 1998, Pérez and Bertoft, 2010).

The wide angle X-ray diffraction pattern allows assessment of the crystalline structure. The diffraction peaks measured with the wide angle X-rays technique are represented as the X-ray diffraction pattern. This pattern also represents the molecular organisation of crystals the spacing between the centre of two amorphous regions and their intervening crystalline clusters. The angle of diffraction indicates the crystal type. The decrease in X-ray scattering indicates an increase in the amorphous region compared to higher diffraction peak from the crystalline zones. The breadth (width) in the X-ray diffraction pattern indicates the crystallite size and the level of the crystallinity is based on the separation and integration of the area under the crystalline and amorphous diffraction peaks. Starch crystallinity ranging from 15-45%, as measured by X-ray, has been reported in native starches (Zobel, 1988a).

The fourth level identified in the starch ultrastructure is the blocklets observed with AFM (atomic force microscope). It is a radial organisation of the lamella into super helix (starch structure size 20-500 nm) (Baker et al., 2001, Vandeputte and Delcour, 2004). Further organisation of the blocklets may result in the growth ring, which is the fifth in the series of starch organisational structure. The growth ring (structure size: 120-500 nm) is a concentric band of materials with a high level of crystalline material that is less susceptible to digestion than other areas. Finally, in the sixth level, these complex architectural assemblies create the stable storage organelle known as starch granules. The granules have characteristic shapes and diameters (2-100 nm) depending on the botanical source. For pigeon pea

granules their size ranges from 8 to 32 μm (Vandeputte and Delcour, 2004). The orientation of the starch structures in a spherical format (growth ring) gives rise to the birefringence that can be observed with polarised light microscopy. The pattern known as Maltese cross is indicative of the starch being in the native form.

In addition to the carbohydrate, other minor components can be present in the starch granule, lipid and protein can occur at low levels. From literature, it was indicated that pigeon pea contains low total lipid (0.13%) and nitrogen (0.02%) (Hoover et al., 1993)

### 2.4.2 Fundamental mechanism of starch thermal transition

Generally, legume starches are considered to have low application due to the poor functionality, which is characterised by low thermal stability of the gel, high level of retrogradation and resistance to shear, digestive enzymes and acid hydrolysis (Hoover, 2010). Therefore, the transformation of legume starch during processing is necessary for appropriate utilisation. Starch transitions occur through the alteration of the levels of organisation within the starch granule and will be influenced by temperature, moisture content and shear. The impact of these physical factors will be dependent on the source of the starch (Hoover, 2010).

The major set of transitions observed for heating starch in water is known as gelatinisation. During gelatinisation, the initial binding of water to the hydrophilic groups soften the amorphous regions of starch granule, and on application of heat, there is disruption of the crystalline region (stripping of starch chains) and loss of helical order. If in excess water, more ingress of water into the granule is followed by irreversible swelling of the granule, leaching of the linear components, an increase in viscosity and the formation of gel on cooling may occur. On gelatinisation the starch granules irreversibly lose their ordered shape and granular integrity, insolubility in water and the original coiling of double helices of the amylopectin.

Gelatinisation is considered an endothermic reaction and much energy is needed to disrupt the crystalline order. The temperature at which gelatinisation occurs is unique to the botanical source of starch. Gelatinisation of starch at concentrations where there is excess water would mean that the granules would swell to several times their original size. When swollen the amylose can easily escape from the swollen starch granules and the granule remnants are easily broken down if sheared. Cereal starches are thought to gelatinise and swell in two stages due to amylose-lipid interactions occurring that inhibit swelling (Ihekoronye and Ngoddy, 1985, Holm et al., 1988, Lai and Kokini, 1990, Morris, 1990, Lai and Kokini, 1991, Morrison et al., 1993, Svensson and Eliasson, 1995, Hoover and Manuel, 1996, Fredriksson et al., 1998, Jacobs and Delcour, 1998, Parker and Ring, 2001, Spigno and De Faveri, 2004, Elliason and Gudmundsson, 2006, Ratnayake and Jackson, 2007, Alcázar-Alay and Meireles, 2015). In summary, starch transitions into native granules follows three steps which are: water absorption by the granule for the increased polymer mobility in the amorphous areas, the loss of crystallinity and finally the loss of intermolecular interactions and granular structure (Ratnayake and Jackson, 2007).

### 2.4.3 State diagram of starch

Starches can be used for many different purposes and how the semi-crystalline material is processed will impact on the transitions and behaviour of the final material. For most starches, temperature and moisture are the main factors affecting starch transitions. Low moisture content could prevent changes in the native starch occurring by not providing sufficient mobility for loss of crystallinity, swelling of granule and amylose solubilisation. The state diagram of starch (Figure 2-8) is a very useful semi-quantitative map depicting how the relationships between starch transition, as a function of temperature and moisture content, affects the physical state of the starch (van der Sman and Meinders, 2011). The

state diagram integrates the concept of water, the glassy state and melting of crystallites in the starch (Slade and Levine, 1988, Slade and Levine, 1995).

Starch can exist as crystallites or amorphous material and will typically exhibit both in the native granule. The amorphous phase can be in the glassy or rubbery state. The transition temperature where material changes from a brittle glass to a soft rubbery material is known as glass transition temperature ($T_g$).

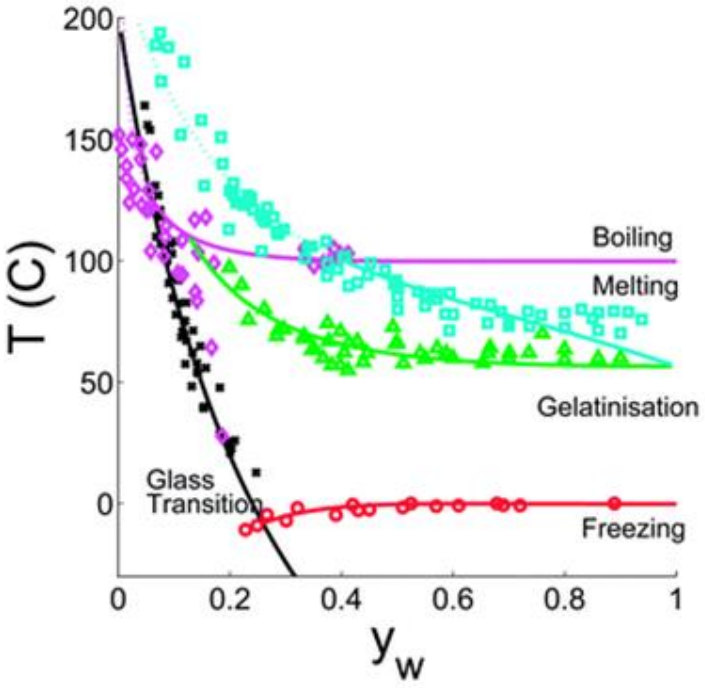



**Figure 2-8:** State diagram of starch transition

Yw: Water content of starch system; T °C: Transition temperature. Source: Van der Sman and Meinders. (2011).

### 2.4.4 Plasticisation of starch by water

High temperatures (>$T_g$) can change a brittle glassy starch material into one that is compliant and deformable. The increased temperature affects

starch polymer backbone chain segmental mobility, and this allows a change in state. A low molecular weight material entering the void volumes associated with the backbone of the macromolecules can also allow additional flexibility and assist in the change from a glass to a rubbery material. The low molecular weight material is known as a plasticiser and the material that plays this role most often for biomaterials is water. The hydration of starch materials can lower the glass transition temperature as indicated for starch in Figure 2-8. During plasticisation, the new rubbery material increases in volume, changes in heat capacity and shows a marked decrease in viscosity when compared to the material in the glassy state.

For starch crystallites to melt, there needs to be sufficient mobility of the surrounding macromolecules to allow for stripping of the chains from the packed crystal and uncoiling of the helices, Therefore, before melting can occur, the starch needs to enter a rubbery state. In starch gelatinisation, the hydration of the amorphous regions allows plasticisation and then the crystalline region can undergo non-equilibrium melting processes (Slade and Levine, 1988). The initial glass transition ($T_g$) will influence the gelatinisation (melting) temperature ($T_m$) of starch, while the total moisture content and moisture distribution within the matrix will be critical factors. The anticipated reduction of $T_g$ may be less than expected when the amorphous regions are closely associated with the crystalline regions due to limiting mobility of the systems due to special limitations (Slade and Levine, 1988, Tester and Debon, 2000).

As indicated in Figure 2-8 the impact of water levels on the melting temperature of starch crystallites is great over the moistures levels associated with dry and soaked pigeon peas. This could be a major factor when cooking as the limited hydration of the pigeon pea could mean that the melting temperatures of the crystallites in the starch are not reached during the processing. For example, if the moisture content in the middle regions of the pigeon pea are less than 20%, temperatures more than 100 °C need to be achieved for the crystallinity to be lost.

The state diagram is also applicable to the re-organisation of the starch macromolecules after they have lost their crystalline structures. If the temperature is below the $T_m$ but above $T_g$ the molecules will assemble to give the lowest energy form, i.e. they return to an ordered crystalline state. This process is known as retrogradation. Although the crystallites formed are not as organised as those in the native structure, amylose, amylose-lipid and amylopectin crystallites can be formed if processed starches are held at the appropriate moisture and temperatures.

### 2.4.5 Starch behaviour during annealing

An additional factor that could be relevant during the soaking and cooking of the pigeon pea is that annealing of the starch may occur. Annealing is the physical modification of starch granule which occur when starch at intermediate (between 40-55%) moisture content is subjected to heat treatment at a temperature (40-75 °C) lower than the starch gelatinisation temperature, but higher than the glass transition temperature. Sometimes annealing occurs unintentionally during production of dried foods from high moisture content material (Jacobs et al., 1995, Tester and Debon, 2000, Jayakody and Hoover, 2008, Adebowale et al., 2009).

In annealing, the helical order is not lost, but there is sufficient plasticisation for the chains in the starch crystal to move for a better organisation of the crystallites and crystalline perfection. During the re-organisations, weaker crystals may disappear Many researchers confirm that annealing leads to an increase in the onset starch gelatinisation temperature. Although, it is often reported that no increase in crystallinity is detected with Wide Angle X-Ray diffraction (WAXS) but with Small Angle X-Ray diffraction (SAXS), annealing may be detected as difference in peak intensity. Other changes in the processing characteristics, such as a decrease in swelling, solubility, pasting properties and water absorption capacities of starches, have been identified following starch annealing. Factors such as starch-water ratio, temperature and time affects the

annealing of starch (Jacobs et al., 1995, Tester and Debon, 2000, Jayakody and Hoover, 2008, Adebowale et al., 2009).

### 2.4.6 Technique for monitoring starch transition

Following starch transition, the alteration in starch structure can be evaluated by different methods (see Table 2-1). These methods, which have been used in the experimental work described in this thesis, are discussed in Chapter 3.

**Table 2-1:** Instrumental techniques for monitoring starch transition.

| Evaluation of starch transition | Instrument |
|---|---|
| Thermal properties | DSC |
| Stirred viscosity during cooking | Rapid Visco Analyser |
| Granular surface | Scanning electron microscope |
| Loss of birefringence | Polarized light microscope |
| Loss of crystallinity | Wide angle X-ray diffractometer |

### 2.4.7 Impact of starch transition on human nutrition

In human nutrition, starch is the major source of energy for the body. Enzymatic degradation of starch is the pathway for the release of the stored energy from starch. Importantly, the digestive enzyme can modify and release energy from starch at a fast rate when the starch has lost its native structure. Therefore, starch conversion by disruption of the crystalline and granular structure during processing becomes a prerequisite for the rapid release of energy from starch during human digestion. Retention or regaining of crystalline order (retrogradation) or physically entrapment of starch impairs human enzymatic digestion and such starches are tagged as being resistant starch. (Holm et al., 1988, Parada and Aguilera, 2009).

Legume starches have been reported to contain high levels of resistant starch. This could be due to the characteristics of the legume starches that result in in-complete loss of order within the starch during processing. However, the way the starch is embedded in the legume seed matrix may impairs the accessibility of the hydrolytic enzymes to the granule.

Sometimes, even when the starch has lost order, as it remains trapped in a cell, it is difficult for the digestive enzymes to penetrate (Ambigaipalan et al., 2014). The resistant starch in legumes could therefore be of two types (1) uncooked starch and (2) encapsulated starch. Much consideration is therefore required in choosing the processing technique that is most appropriate for the required transition of the starch and for its adequate digestion.

## 2.5 Changes in protein when cooked

### 2.5.1 Understanding the native structures of protein

Proteins are macromolecules with polypeptides chains formed through peptide bonds that link amino acid residues (Sikorski, 2001b, Cozzone, 2010). Among food plants, legumes have the highest protein content. The structure function relationships of food protein influence their application. The structures formed through the various interactions of amino acid residues during protein formation determines the unique physicochemical characteristics of the protein. Depending on the interactions of different amino acid residue, subunits and polypeptides there exists four structural levels of protein namely, primary, secondary, tertiary and quaternary. (Milewski, 2001).

### 2.5.2 Pigeon pea seed storage protein compared with cereal

The protein content of pigeon pea is reported to be about 23%. Similar to other legume seeds, seed storage proteins are the major source of protein in pigeon pea. These storage proteins functions as a reserve nutrient necessary for the growth of seed. Microstructurally, legume proteins can be observed as spherical object measuring 1-3 μm. These protein bodies are within the protein matrix that embeds the starch granule. Within the protein mass, there are crystalline inclusions that are rich in soluble phytate salts called phytin (Lluch et al., 2001). According to their solubility, pigeon pea proteins are grouped into four fractions (Table 2-2): globulin; salt soluble,

albumin; soluble in water and diluted salt, prolamin; soluble in water-ethanol solution, and glutelin; soluble in acid/alkali (Singh et al., 1990, Saxena et al., 2010). The globulin and albumin fractions influence the functionality of legume protein. Like the majority of pulses and oilseeds, globulin (separated into 11S: legumin and 7S: vicilin) predominates in pigeon pea with over 60% weight (Table 2-2). Storage proteins are oligomeric (2 or more subunits), bound by combination of intermolecular disulphide bond, hydrogen bonding, ionic bonding and hydrophobic bonding (Bewley and Black, 1994, Lampart-Szczapa, 2001).

**Table 2-2:** Pigeon pea seed protein fractions.

Protein fractions of dhal sample of high and normal protein genotypes

| *Genotype* | *Protein (g per 100 g)* | *Albumin* | *Globulin* | *Glutelin* | *Prolamin* | *Total* |
|---|---|---|---|---|---|---|
| | | *(g per 100 g protein)* | | | | |
| HPL 8 | 28·7 | 9·1 | 63·5 | 20·2 | 2·9 | 95·7 |
| HPL 40 | 31·1 | 8·0 | 66·2 | 19·7 | 3·2 | 97·1 |
| C 11 | 24·8 | 7·7 | 60·5 | 23·3 | 3·6 | 95·1 |
| ICPL 211 | 23·1 | 8·6 | 60·3 | 22·8 | 2·1 | 94·5 |

Source; Singh et al., (1990).

**Table 2-3:** Protein composition of cereals for comparison with pigeon pea seed protein in Table 2-2.

| Cereal | Protein content (%) | Albumin (%) | Globulin (%) | Prolamin (%) | Glutelin (%) |
|---|---|---|---|---|---|
| **Wheat** | 10.6 | 9 | 5 | 40 (gliadin) | 46 (glutenin) |
| **Maize** | 9.8 | 4 | 2 | 55 (Zein) | 39 |
| **Barley** | 11 | 13 | 12 | 52 | 23 |
| **Oats** | 9.3 | 11 | 56 | 9 | 23 |
| **Rice** | 7.3 | 5 | 10 | 5 (oryzin) | 80 (oryzein) |
| **Sorghum** | 8.3 | 6 | 10 | 46 | 38 |

Source: Haard et al., (1999).

Table 2-2 shows the proteins fractions in pigeon pea while **Table 2-3:** Protein composition of cereals for comparison with pigeon pea seed protein

in Table 2-2. In wheat and majority of cereals, glutelin is most abundant fraction of the seed protein followed by prolamin. Globulin is more predominate in oat, constituting 56% of the storage protein. The structural characteristics of the storage proteins that occur in the pigeon pea could therefore be expected to impact on the proteins' functionality (solubility, water holding and binding capacity) and influence the quality of pigeon pea products (Lampart-Szczapa, 2001, Sikorski, 2001a).

### 2.5.3 Changes in the protein structure during processing

Changes in native protein are reflected in the various roles it plays in a food system. These changes are mainly induced by heat, pH and mechanical stress with impact on the functional, textural, rheological and microstructural characteristics of the food. The processes involved in the alterations of protein structure in a food system includes denaturation, aggregation, coagulation and cross-linking. Both aggregation and coagulation are similar and can occur simultaneously during product formation without differentiation. Some researchers suggested that due to the structure, conformation of seed storage protein, that these proteins undergo limited denaturation, and that most legume protein remains soluble after cooking. If heat induced insoluble aggregates are formed, then their enzymatic breakdown would be impaired and so would be the release of amino acids during digestion(Bewley and Black, 1994, Lampart-Szczapa, 2001).

### 2.5.4 Denaturation of protein

Denaturation is the unfolding of the native conformation of a protein (secondary, tertiary or quaternary protein structure) without rupture of the peptide bonds. It can occur by exposure of the protein to high temperatures, extreme acidic or alkaline pH, chemical agents or shear and has the consequence of exposing the native primary structure and can result in loss of biological activity. In some cases, denaturation induces

coagulation or formation of precipitates. The added stability of protein at its isoelectric point impairs denaturation. The isoelectric point of Pigeon pea protein ranged from pH 8.5-12.5 (Mwasaru et al., 1999). To ascertain the extent of denaturation, the protein solubility in different aqueous solution is typically determined (Ma and Harwalkar, 1988b, Boye et al., 1997, Meng and Ma, 2001, Milewski, 2001).

#### 2.5.4.1 Heat denaturation of protein

Since the mobility of biomaterials is increased by heating, heat can induce alterations within native protein structures so that they are denatured. When protein is heated above a critical temperature, most of the hydrogen bonds in protein structure that requires higher kinetic energy are disrupted together with non-polar hydrophobic and electrostatic interaction in the tertiary structure. The conformation entropy of the protein chains increases as the temperature increased which causes the unfolding of the native protein structure. The disulfide and peptide bonds are not normally broken by heat. The transition temperature at which the denaturation occurs is known as denaturation temperature (Damodaran, 2007). The denaturation thermal properties can be measured using differential scanning calorimeter (DSC). The different transition temperatures are related to the stability of the different domains of protein molecule (MA and Harwalkar, 1988a). While the glass transition temperature ($T_g$) and the denaturation temperature ($T_d$) of protein is decreased as moisture content increases, the enthalpy of denaturation (the flow of heat for denaturation) increases (MA and Harwalkar, 1988a). Due to the thermal denaturation, the functional properties of native proteins are affected but positives benefits from heat treatment can be that protein based anti-nutritional factors, such as trypsin inhibitors and lectins, are destroyed.

## 2.6 Extrusion cooking

Due to the thermoplastic properties of starch, in which starch crystalline structures can melt and soften when heated and hardened on cooling, it is possible to create varieties of ready-to-eat snacks from starch. A process method to achieve this is by a thermomechanical extrusion, similar to that used for synthetic polymers. Thermomechanical extrusion is a short time (5-200 second) continuous cooking at high pressure (above the vapour pressure of water) that combines moisture (10-25%), high temperature (above 100 °C) and mechanical shear (250-500 rpm screw speed) for the development of uniform food products, especially expanded (puffed) snacks and textured foods. Generally, such a process is referred to as high temperature short time cooking (HTST). It is an efficient low-cost production technique involving mixing, shearing, cooking and forming (Harper, 1981, Doublier et al., 1986, Lai and Kokini, 1991, Moraru and Kokini, 2003, Ferreira et al., 2011, Moscicki, 2011, Robin et al., 2014).

The extrudate (product from extrusion cooking) is influenced by extrusion variables (screw speed, temperature, moisture content, torque, SME, die pressure and diameter) and different raw materials characteristics (high or low calorie, high protein and fibre content). In the past, most of the extruded snacks (biscuits, crisps, bread, cheese balls, tortillas and cereal bars) were made from cereals and root based starches but recently, due to consumer's choices, products from sources with high fibres and legume materials are being developed (Berrios et al., 2004, Robin et al., 2012, Robin et al., 2014). The benefit of the extrusion process includes increased starch and protein digestibility, destruction of anti-nutritional factors, increased soluble dietary fibre and reduction of lipid oxidation. In addition to sensory evaluation, the impact of extrusion on product quality is usually assessed by the degree of expansion, hardness, interactions with water and investigation of the microstructure (Lin et al., 1997, Liu et al., 2000, Anton et al., 2009, Ferreira et al., 2011).

### 2.6.1 Mechanism of product formation during extrusion

Starch conversion during extrusion is the major factor that impacts on product expansion and puffing. The transition of starch at adequate extrusion conditions (12-16% moisture content, 90-164 °C and shear), would result in the melting of the crystalline structure. Due to an increase in mobility of starch chains and conversion of starch as the continuous matrix, the extruding material behaves like a molten polymer exhibiting a viscoelastic properties (Lai and Kokini, 1991). This starch transition is dominated by the shear forces, which tear apart the granule, causing the disruption of molecular hydrogen bonds and faster transfer of water into the interior starch molecules. This transfer of water re-establishes new hydrogen bonds between water and the amorphous starch chains and hence enables starch chains to flow as an amorphous melt (Lai and Kokini, 1991). The levels of starch breakdown (starch conversion) that can occur in the extruder are much greater than that typically achieved in thermal processing associated with gelatinisation in high water environments.
The screw design forces the melt towards the die and the creation of the high temperature and pressures, results in water becoming superheated. However, on exiting from the die to ambient conditions, a pressure difference will cause steam evaporation (flash-off). The resulting bubble growth will make the product expand and an open foam structure can occur. However, if the starch structure does not move from a rubbery to glassy state on cooling of the expanded product, the structure can contract, and the bubbles will collapse to form a solid closed porous extrudate (Lai and Kokini, 1991, Kokini et al., 1992, Moraru and Kokini, 2003, Robin et al., 2011)

Extruded products can make snacks that are directly or indirectly expanded. Direct expansion occurs during the exit of the melt from the die. These products are often called second generation snacks. While in third generation snacks the extrudate emerges from the die still in a rubbery state and does not expand. These extrudates are often formed as pellets

and once dried to an optimum moisture content pellets are expanded by a re-heating treatment (frying, direct heating or microwaving). Many snack products, ready to eat breakfast cereals and animal feeds are made by these thermomechanical extrusion techniques.

### 2.6.2 Types of extruders

Extrusion cooking is carried out with extruders which are typically defined according to the screw type (Figure 2-9). Extruder has a barrel that contains screws and their design defines three basic sections (feeding, kneading and metering). The feed section continuously receives the solid raw material into the extruder. In the transition section, the material is compressed and kneaded followed by the dissipation of mechanical energy. The dissipation of energy increases the material temperature and transforms (cooked) the material to a plasticised viscous mass. In the metering section, the molten mass is compacted before being pushed through the die. However, mixing is limited in the single screw and slippage will occur in samples at higher moisture or if they contain too much oil.

With the single rotating screw inside a tight-fitting barrel, the heat transforms the extruding material into a viscous mass. (Harper, 1992, Tayeb et al., 1992, Moscicki and Zuilichem, 2011). The single screw thermomechanical extruder may operate with a pre-conditioner (heating and hydration of the feed before entering the extruder barrel) to increase the residence time and reduce the mechanical power consumption. The barrel of the extruder may also be heated.

Twin-screw extruders are often now used for industrial extrusion processes due to their higher mixing capacity and ability to use different raw materials (both viscous and hard to break material). These extruders consist of two types of screw configurations (co-rotating and counter-rotating) according to the direction of the screw rotation. In co-rotating, which are most typical, the screws rotate in the same direction. Melting and material transformation occur to the greatest extent when flow restriction is present.

With the option of assembling the screw elements on the shaft, extrusion processes are easy to tailor to form the product required. A picture of the pilot scale twin screw extruder used in the research reported in this thesis is shown in

**Figure 2-10**.

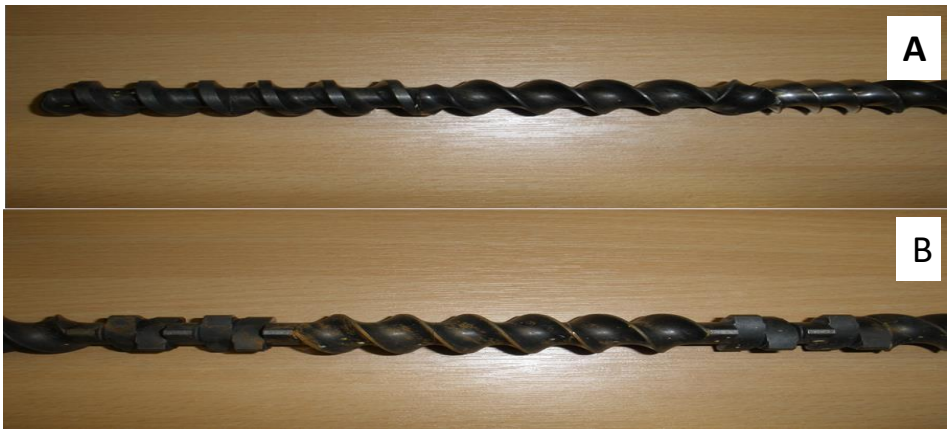



**Figure 2-9:**Examples of screws (A) low shear screw (B) high shear screw from the experimental twin screw (TSE24 Thermo Scientific, Germany).


**Figure 2-10:** Experimental twin screw extruder with barrel section opened.

The intermeshing flights of the screw enables materials to be conveyed forward without being locked in the space between the surface of barrel and the screw. The general screw configuration enhances mixing and easy handling of sticky materials (Harper, 1992, Raiz, 2001, Moscicki and Zuilichem, 2011, Steel et al., 2012). Specialised flight elements on the screws provide specialised mixing function or additional shear. Figure 2-9 shows two screws designed with one having mixing and reversing flights to increase the shear experienced by the melt. In all screw configurations, the most important factor is that there should be sufficient frictional forces to convey and to convert the extruding material into a viscous mass. (Moscicki and Zuilichem, 2011).

### 2.6.3 The effect of extrusion conditions on starch conversion

During extrusion, the starch structures could be transformed through gelatinisation, melting and fragmentation (Lai and Kokini, 1991). Choosing the appropriate extrusion conditions and material are essential to enhancing the conversion of native starch and denaturation of protein. At low moisture extrusion (<15% moisture content) and high temperature, the lubrication of the melt may be insufficient, causing an increase in mechanical energy as flow will be impaired. Inversely, in high moisture extrusion (<30% moisture content), less friction occurs between the extruding material and the extruder barrel and screws leading to poor formation of melt and low dissipation of energy (Forte, 2011). Lai and Kokini, (1991) reported that at high shear forces, occurring when moisture content is low (12-16% wet basis) and there are high die temperatures, the degree of starch conversion is high. Therefore, depending on the material composition, manipulation of processing variables such as screw speed, residence time, feed rate and moisture content can impact on extrudate structures and texture (Lai and Kokini, 1991).

### 2.6.4 The effect of fibre inclusion on extrusion

The use of high fibre materials during extrusion has been on the increase due to the health value of the product.(Ferreira et al., 2011, Robin et al., 2012). Incorporation of fibre can pose some difficulties during extrusion if the concentration is not controlled. High concentration (>30%) of mostly insoluble fibre can result in a compact high bulk density product due to the low physicochemical compatibility (disruption of structure) between insoluble fibre and the carrier material which is typically starch. The fibre increases the melt viscosity and causes early collapse of the expanding bubbles (Robin et al., 2012). However some addition of soluble fibre was reported to lead to an increase in expansion, as this reduced the shear viscosity of the melt and acted as nucleating agents for bubble expansion (Pai et al., 2009).

#### 2.6.4.1 The impact of extrusion on protein structure

The behaviour of protein during extrusion will be dependent on the native structure. Globulin protein structure may be initially denatured and texturised during extrusion. In addition to the denaturation, protein chains are stretched with macromolecular alignment in the direction of flow (depending on the shear rate and melt viscosity) that results in the forming of solid product on cooling. The texturisation of protein during extrusion cooking increases its application. However, for most globular proteins, due to its aggregation within the conditions occurring in the barrel of the extruder, no homogenous melt occurs and protein solubility decreases. Loss of protein solubility is detrimental to digestibility as it impairs the release of amino acids to the body (Valle et al., 1994, Camire, 2001, Forte, 2011, Steel et al., 2012).

## 2.7 Understanding effective processing of pigeon pea

Pigeon pea like some other legumes may have great potential due to its physico-chemical properties section 2.1.2. Limitations in the conventional utilisation of pigeon pea have been based on several factors including the presence of some anti-nutritional factors (section 2.2.), and difficulties in the hydration of seed components (seed coat and cotyledon). Hence the long soaking duration and the prolonged cooking times are required. It is generally considered that the prolonged cooking times is a defect acquired from physiological activities during post-harvest storage at tropical conditions. If this material is to fulfil its possible potential, then overcoming its' difficult to cook characteristics is essential. A clear understanding of the prevalence of this hard to cook feature and how this "difficult to cook" material behaves during processing is important. The interventions to negate this quality will then be tested.

The transformation of whole pigeon pea seed into ready to eat snacks could be a positive action for meeting the food and nutritional security of developing nations and people affected by food related diseases (diabetes, cardiovascular and digestive diseases). However, the physicochemical characteristics and constrains of pigeon pea need to be carefully considered. A method that can impart major degradation of chemical components, disrupt structures with temperature, and shear is thermomechanical extrusion. This relatively cheap processing technique could be appropriate to meet the objectives of creating an interesting snack product from pigeon pea, which has suitable textural characteristics and no harmful anti-nutritional factors. With this goal an extrusion method needs to be developed and the products tested to build an understanding of how a direct expansion technique may be used in the production of second-generation snack (characterised as ready to eat and low bulk density product) from a variety of Nigerian pigeon pea.

# 3 Materials and Methods

## 3.1 Materials and chemical

Three Nigerian commercial varieties of pigeon pea namely white Agatu, red Agatu and Bayelsea were initially used but later White Agatu was selected as research pigeon pea based on the results reported in chapter 4. The white and red Agatu pigeon pea (5 months post harvested) were bought from a local farm in Agatu, Benue State, Nigeria, while the Bayelsa pigeon pea was bought from a local market (Eke Awka) in Awka, Anambra state, Nigeria. They were stored in a dry store at 20°Cuntil required for use. The chemicals used during this research are detailed along with their methodology in the appropriate section. All chemicals were laboratory standard grade.

## 3.2 Methods used in seed treatments

Many factors were considered in selecting the methods for the seed treatment in the study of the processing characteristics of pigeon pea. The methods were chosen to improve seed hydration and softening. Some of the methods were based on the modification of conventional hydration techniques and what is found in the literature. Other methods were selected to optimise the disruption of cellular components during processing.

All the milled seed powder used in this study was either prepared from the native or treated pigeon pea seed. In all instances, the milled seed powder was prepared for use as the major feed material for the production of extruded snacks from pigeon pea. The milled seed powder from the treated seeds was targeted to be more hydrated and plasticised during the treatment period compared to the short period of hydration during extrusion cooking. It is also expected that the modification that will occur in the hydrated and softened seeds will produce high quality (Nutritional

and structural stable) snacks compared to the non-treated native milled seed powder.

However, the seed treatments applied in this research were conventional soaking, dehulling, hydration at moderate temperature (below starch endset gelatinisation temperature) and cell wall degradation involving hydrothermal, alkaline and enzymatic treatment. The treated seeds were either dry-milled or wet milled into powder. The native pigeon pea seed, which is the non-treated seed, was also milled and served as the control throughout the research. Milled samples were stored at 4°C until use for either analysis or extrusion processing.

### 3.2.1 Conventional soaking and dehulling

The conventional soaking method was adopted from the traditional soaking method for pigeon pea in Nigeria at ambient temperature. It involved soaking of 500g seeds from three varieties of pigeon pea (white Agatu, red Agatu and Bayelsa) in 2 L of tap water in a separate beaker at 20 °C for 12 hours. To obtain the soak and dehulled samples (CSD), the soaked samples were manually dehulled. A triplicate of all samples was obtained. Both the conventionally soaked (CS) and soaked and dehulled (CSD) seeds were dried and milled according to section 3.2.3.4. The samples were stored at 4 °C until evaluation of seed physicochemical and pasting properties. Results of evaluation are discussed in chapter four.

**Table 3-1:** Codes for native and conventional soaked samples.

| Pre-treated sample code | Sample description |
|---|---|
| WA-NS | white Agatu-native seed |
| RA-NS | red-Agatu -native seed |
| B-NS | Balyelsa-native seed |
| WA-CS | white Agatu-conventionally soaked and dried |
| RA-CS | red-Agatu -conventionally soaked and dried |
| B-CS | Balyelsa-conventionally soaked and dried |
| WA-CSD | white Agatu-conventionally soaked, dehulled and dried |
| RA-CSD | red Agatu-conventionally soaked, dehulled and dried |
| B-CSD | Balyelsa-conventionally soaked, dehulled and dried |

### 3.2.2 Seed hydration and softening treatment

#### 3.2.2.1 Hydrothermal treatment(HTT) at 37, 50, 70 and 90°C

To monitor the water absorption behaviour of pigeon pea seed during soaking and maximise softening of seed, the conventional ambient soaking temperature was modified to 37, 50, 70 and 90 °C. This method was adopted from Singh and Kulshrestha (1987) with modification of temperature and soaking time. The white Agatu pigeon pea, which was selected for the main PhD research, was used in this hydrothermal treatment (HTT). Native seed (500 g) in triplicates of the sampling was placed in a glass beaker containing 2 L of reversed osmosis (RO) water and soaked at 37 °C, 50 °C, 70 °C and 90 °C for 4 h using a water bath without stirring (quiescent environment). Samples were collected after 30 min intervals by removing three beakers (representing triplicate samples) until the end of the 4 h and the seed water uptake (percentage moisture content) was determined. The percentage moisture content was further used to plot the water uptake curve and determine the hydration characteristics. The steep water conductivity and seed deformation force of all the hydrated samples (all temperatures and time interval inclusive) were determined according to sections 3.4.1.5 and 3.4.4. All other analyses (pasting,

thermal properties and microstructure were carried out after wet and dry milling of the (37 °C, 50 °C, 70 °C and 90 °C) hydrated seeds sampled at intervals of 2 h and 4 h. The wet milling consisted of milling the hydrated seeds in a coffee grinder (section 3.2.3.2). While the dry milling consisted of milling seed dried (3.2.3.1) after drying the hydrated seed at 40 °C for 24 h using a vacuum dryer. Samples were named according to soaking temperature (°C), time in hour (h), WM: wet milled or DM: dry milled. The results of this investigation are discussed in chapter five.

#### 3.2.2.2 Preparation of native pigeon pea (NPP) powder for cell wall degradation treatment

Native pigeon pea seed (1 kg) was dry milled according to section3.2.3.1. From the milled flour, 500 g of flour was kept as control flour (NPP) while another 500 g was mixed with 125 g tap water for 10 min to produce the native wet milled powder. The native dry milled powder serves as control to monitor the effect of the cell wall degradation.

#### 3.2.2.3 Hydrothermal and alkaline treatment targeting cell wall degradation

The hydrothermal treatment was carried out using native white Agatu pigeon pea seed weighing 500 g. The treatment involved mild shear stirring and steaming of the seed under vacuum with 5 L tap water at 90 °C in a 15 L capacity jacketed process vessel (Roboqbo) for 5 min. The mild shear was obtained through setting the equipment paddle speed at 40 rpm. The alkaline treatment was conducted in the same way except that tap water was replaced with 0.5% sodium bicarbonate solution (pH 8.5). The treated seeds were drained of the steep water before wet milling at in the jacketed vessel (section3.2.3.3) at 25 °C. The samples were portioned for enzymatic treatment 3.2.2.4) and freeze drying according to section 3.2.3.6. Although samples were labelled according to Table 3-2 but only the wet milled were used for enzymatic treatment and the freeze-dried samples were used for further analysis.

**Table 3-2:** Sample code for the hydrothermal and alkaline treatment targeting cell wall degradation.

| Sample | Sample description |
|---|---|
| HTT | hydrothermally treated |
| BB | alkaline(bicarbonate) treated |

### 3.2.2.4 Enzymatic treatment

Enzymatic treatment of samples from Table 3-2 was carried out with cell wall degrading enzyme (Viscozyme L, Novozymes Denmark) produced from a selected strain of *Aspergillus aculeatus*. It contains a wide range of carbohydrases, which includes arabanase, cellulase, β-glucanase, hemicellulase, and xylanase with an activity of 100 fungal beta glucanase per ml. Based on the enzyme application manual, the enzyme optimum pH of 4.7 was achieved by reducing the pH with sodium acetate at pH 5.

Following the pH adjustment, the enzyme measuring 0.5% (W/V) of the sample weight was added to wet milled samples listed in Table 3-2 and the native wet milled pigeon pea powder (NPP) from section 3.2.2.2.The enzymatic incubation was carried out at 50 °C for 60 minutes (the manufacturers' recommended optimum temperature and minimum holding time) in the jacketed process vessel (Roboqbo) under atmospheric pressure (700 mbar) and at paddle speed of 30 rpm (speed was set to avoid increase in incubation temperature due to frictional force). At the end of the 60 min enzymatic incubation, the deactivation of the enzymatic process was performed by increasing the jacketed vessel temperature to 60 °C for 5 min since the optimum activity temperature range was 40-50 °C. All the enzymatic treated samples were freeze dried (section 3.2.3.6), milled and labelled Table 3-3.

**Table 3-3:** Samples' description code following enzymatic treatment.

| Sample | Sample description |
|---|---|
| NPPZ | native wet milled pigeon pea + enzymatic treatment |
| HTTZ | hydrothermal + enzymatic treatment |
| BBZ | alkaline(bicarbonate) and enzymatic treatment |

The impact of the enzymatic treatment on the functionality of the milled powder such as water absorption, water solubility capacity, swelling power, pasting, and DSC thermal properties were used in selecting the most appropriate flour for extrusion cooking. The results from this investigation are discussed in chapter five.

### 3.2.3 Additional preparation of the seed sample

#### 3.2.3.1 Dry milling and particle size analysis

The native (non-treated), treated and dried seeds were milled using the lab disc mill (Lab mill 3600, Perten Instrument, Sweden). The mill was initially set at 3 for pre-milling and finally re-set to 1 for final milling to obtain the powder. The particle size of the dry milled powder was determined with a mechanical sieve shaker (AS 200 control, Retsch, Germany) in vibratory motion and amplitude of 1.5mm. The particle size of the dry milled powders ranged from 125 to 710 µm with majority (60%) between 125-355 µm.

#### 3.2.3.2 Wet milling with Kenwood blender and coffee grinder

The hydrated and drained seed from 37 °C -90 °C hydrothermal treatment (HTT) was wet milled for 10 min using a Kenwood blender. To further reduce the particles, the wet milled powder from the Kenwood blender was milled with a coffee grinder (De'Longhi Deluxe-KG49). Due to the high moisture, the particle size of the wet milled samples was not determined with the Test sieve shaker.

#### 3.2.3.3 Wet milling with jacketed vessel

The treated seeds from short time hydrothermal (90 °C, 5 min) treatment in the jacketed vessel (Ql15L, Roboqbo, Italy) were wet milled following the

draining of steep water. The seeds were wet milled for 60 secs using the jacketed vessel set at 25 °C and blade speed 2500 rpm.

#### 3.2.3.4 Convection oven drying

Samples were dried with a convection oven drier (ED 56, Binder, Tuttlingen, Germany) at 50 °C.

#### 3.2.3.5 Vacuum drying

Samples were dried for 24 hours using a vacuum oven drier (Gallenkamp OVL 570 010J by Weiss Technik, UK) set at 40 °C.

#### 3.2.3.6 Freeze drying

Samples were dried using a freeze dryer (1311-03/08 JM, Edwards-Modulyo, Edwards, UK) set at -40 °C and vacuum pressure at 0.07 bar.

#### 3.2.3.7 Centrifugation

An induction drive centrifuge (Beckman J2-21M) was used for the centrifugation of the sample at more than 3000 g while a Jouan CR 31 multifunction Thermo Electron Corporation, USA was used for centrifuging samples in a 15 ml screw cap at 40 °C, 3000 g and 10 min.

## 3.3 Thermomechanical extrusion process

### 3.3.1 Feed preparation for thermomechanical extrusion

Different extrusion feed materials were prepared from white Agatu pigeon pea based on the hydration limitations of native pigeon pea. A preliminary extrusion of dry milled native (non-treated) pigeon pea powder was carried out after addition of water to the feed in the extrusion barrel to obtain three levels of moisture content (low:12% and 14%and high:22%). The feed moisture level is inclusive of 11% moisture content of dry milled powder.

The majority (more than 60%) of the milled pigeon pea powder consists of particle size measuring from 125-350 µm. The sample codes and the extrusion conditions for the preliminary extrusion is in Table 3-4.

However, a second set of feed material were prepared from wet and dry milled hydrothermally and alkaline treated pigeon pea seeds according to the procedure in section 3.2.2.3 and flow chart in Figure 3-1.The wet and dry milled had moisture contents of 22% and 11% respectively. However, the native milled powder (NPP)was also conditioned at ambient temperature (20 °C) to high moisture content (22%) to serve as control flour during the extrusion cooking. Hence a total of 6 samples shown in Table 3-5 were used for the second batch of the extrusion cooking.

**Table 3-4:** Sample codes and extrusion conditions for the high and low moisture preliminary extrusion of native pigeon pea (NPP).

| Extrudate Code | Die temperature (°C) | Screw speed(RPM) | Feed rate(kg/h) | Feed moisture content (%) |
|---|---|---|---|---|
| E1 | 120 | 200 | 8 | 22 |
| E2 | 120 | 300 | 8 | 22 |
| E3 | 120 | 400 | 8 | 22 |
| E4 | 140 | 200 | 8 | 22 |
| E5 | 140 | 300 | 8 | 22 |
| E6 | 140 | 400 | 8 | 22 |
| E7 | 150 | 200 | 8 | 22 |
| E8 | 150 | 300 | 8 | 22 |
| E9 | 150 | 400 | 8 | 22 |
| E10 | 150 | 200 | 8 | 14 |
| E11 | 150 | 300 | 8 | 14 |
| E12 | 150 | 400 | 8 | 14 |
| E13 | 150 | 400 | 8 | 12 |

**Table 3-5** Samples' code for the extrusion of treated and non-treated (native) feed material (MC = moisture content).

| Extrudate code | Description of feed material |
|---|---|
| NPPHME | high moisture (22%) native pigeon pea |
| HTTHME | hydrothermally treated pigeon pea + wet milled (22% MC) |
| BBHME | steamed bicarbonate (alkaline) pigeon pea+ wet milled (22% MC) |
| NPPE | dry milled native pigeon pea (11% MC) |
| HTTE | hydrothermally treated pigeon pea + dry milled (11% MC) |
| BBE | Steamed bicarbonate (alkaline) pigeon pea+ dry milled (11% MC) |

White Agatu
Pigeon pea seed

Dry milling

NPPE
(11% moisture content)

Mixing with $H_20$ at ambient condition (20 °C)

NPPHME
(22% moisture content)

Alkaline steam treatment
(0.5% Sodium bicarbonate) in jacketed vessel @ 90 °C, 5 min

Hydrothermal steam treatment in jacketed vessel
@ 90 °C, 5 min

Draining

Hydrated seed

Wet milling

Drying & milling

BBHME
(22% moisture content)

BBE
(11% moisture content)

Draining

Hydrated seed

Wet milling

Drying & milling

HTTHME
(22% moisture content)

HTTE
(11% moisture content)

**Figure 3-1:**Flow chart for the preparation of treated and non-treated feed material for extrusion

### 3.3.2 Extruder Configuration

Extruded products were developed with a twin screw extruder (Prism TSE 24 MC, Thermo Scientific, Germany) with a die of 4 mm diameter and 5 cm length, screw length/diameter ratio of 40 (diameter: 22.5 mm, length:980 mm), motor power 5.5 kW, output 50 kg/h. The shafts of the two co-rotating intermeshing screws were configured into three sections (feed, compression and metering) as follows: F60 degree conveying and mixing elements (mixing element with kneading block) with serial configurations: 30 x conveying flights (each 25mm long),6 x mixing elements (each with a width of 5mm), 3 x conveying flights,7 x mixing elements, 2.25 x conveying flights and finally 1 x extension end flight.
The extruder barrel was segmented into nine temperature controlled zones as shown in Table 3-6.

**Table 3-6:** The configuration and temperature zone of extruder barrel.

| zones | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| Temp (°C) | 30 | 30 | 40 | 40 | 50 | 60 | 80 | 100 | 120 |
| activities | feeding | feeding | mixing | mixing | mixing | mixing | kneading | Kneading | metering |

### 3.3.3 Development of extruded products

Varieties of extruded products were developed in batches (preliminary high and low moisture extrusion and the treated and non-treated extrusion)using the different feed materials in 3.3.1 in addition to the barrel temperatures in Table 3-6, extrusion conditions in Table 3-4and Table 3-5. As previously stated, the feed materials were extruded using a twin screw extruder (Prism TSE 24 MC, Thermo Scientific, Germany). The feed materials were fed through the hopper of the extruder after equilibration of

the configured extruder. Following the mixing (with or without water) in the barrel, the feed material was compressed and kneaded with the aid of kneading blocks for the formation of a homogenous melt. With the build-up of pressure during the flow of the melt, the melt was further pressurized to pass through a die of 4mm to the atmospheric condition. Following the expansion and cooling of the extruded melt at atmospheric conditions, extrudates were developed and cut into lengths. An average residence time of 22 seconds was recorded during the extrusion. Varieties of extruded products were developed due to the different feed materials and extrusion conditions. The extrusion process is further illustrated with flow chart shown in Figure 3-2. Extrudates were labelled according to sample code in Table 3-4 and Table 3-5. Following the labelling, the extrudates were either air or oven dried according to the moisture content, before storing in air tight containers for further analysis. The results from the two preliminary studies were used to optimise the extrusion involving treated feed material.

Pigeon pea seed
(Pre-treated /native)

Milled whole pigeon pea
(feed material)


Twin screw extruder for extrusion cooking

Extruded snack food from pigeon pea

**Figure 3-2:** Flow chart for the extrusion of pigeon pea.

## 3.4 Analysis of physico-chemical properties

### 3.4.1 Physical characteristics of the seed/ milled powder

Seed weight, length and thickness were measured to characterise the three commercial pigeon pea varieties following the published method (Güzel and Sayar, 2012). The weight of 100 seeds was measured with analytical balance while a digital Vernier Calliper (Mitutoyo, Japan) was used to measure the thickness and length of 25 replicate seeds

#### 3.4.1.1 Determination of the water uptake by the seed coat and cotyledon

To investigate whether most of the water absorbed by the seed goes to the seed coat or cotyledon, a water uptake test was conducted. To 30 g seeds in two triplicate 250 ml glass beaker, 80 ml of water was added and inserted into a water bath (Fisher Brand FB80307, Grant Instrument Ltd Cambridge) without stirring at 50 °C for 60 min. The seeds were blotted to remove surface water and the weight of wet seed determined. The water absorbed by the seed was calculated as the weight of the dried seed subtracted from the weight of the soaked seed. To determine the water absorbed by the seed coat and cotyledon, the seed coat of hydrated seeds was peeled so the weight of both seed coat and cotyledon could be determined on a wet weight basis (wwb). These seed components were further dried in a convection oven(section3.2.3.4) for the determination of the moisture content on dry weight basis.

#### 3.4.1.2 Determination of bulk and specific density of the seed

A known weight of pigeon pea seed (P) was used to fill a 250 ml weighed plastic cylinder (C). The individual and combined weights of the seed and cylinder were recorded (C+P) before the known volume of water was used to fill in the air space between the seeds in the cylinder. The combined weight of seed and water in the cylinder was recorded (P+W+C). The bulk

density and specific density of the seeds were calculated using equation 1 and 2.

$$\text{Bulk density (g/ml)} = \frac{\textbf{weight of the seed}}{\textbf{volume of cylinder}} \quad (1)$$

$$\text{Specific density (g/ml)} = \frac{\textbf{weight of the seed}}{\textbf{real volume of cylinder occupied by the seed}} \quad (2)$$

Real volume of the cylinder occupied by the seed = volume of cylinder (250ml) –volume of water that filled the cylinder with the seed

Volume of water that filled the cylinder with the seed = (P+W+C)-(P+C)

### 3.4.1.3 Hydration capacity of seed

The hydration capacity of the conventionally soaked seed in section 3.2.1was calculated with equation 3 according to Fasoyiro et al. (2010) and Nassar Abbas et al., 2008. The hydration capacity measured the amount of water (wet weight basis) absorbed by the seed during soaking at ambient condition and was calculated with equation (3) as the percentage of water absorbed by 100 g whole seed after soaking in water for 12 h at room temperature (20 °C) over the seed weight before soaking.

$$\text{Hydration capacity (\%)} = \frac{\text{weight of water in soaked seed} \times 100}{\text{wt of seed before soaking}} \quad (3)$$

Where the amount of water in the soaked seed was calculated as the weight of the soaked seed minus the weight of dry seed before soaking.

### 3.4.1.4 Moisture content in dry weight basis (dwb)

Determination of moisture content (dwb) of the hydrated seed, seed flour and ground extrudate were determined according to AOAC 2000 method. Apart from using this method in calculating the moisture content of the milled seed and extrudate powder, it was also used in calculating the amount of water absorbed by the seed after the hydrothermal treatment

(HTT) at 37-90 °C. The moisture content of the hydrated seed in dwb is equivalent to the water uptake of the seed during the hydration treatment. Before determining the moisture content of the hydrated seed, the surface water on the seed was thoroughly blotted with blue paper tissue.

To determine the moisture content, 2-2.5 g (W1) of the sample was weighed into a previously dried and weighed aluminium dish and oven dried at 105 °C in a convection air forced drier (Binder, Germany). The hydrated seed and wet milled seed powder were dried for 24 h while dry milled seed flour and extrudates were dried for 8 h. After drying, the sample was cooled and re-weighed (W2). The moisture content was calculated with equation (4) as the percentage weight loss over the initial sample weight.

$$\text{Moisture content (\%)} = \frac{(w1 - w2) \times 100}{w1} \quad (4)$$

#### 3.4.1.5 Electrical Conductivity

The electrical conductivity (EC) of the HTT seed soaking solutions was determined with an electrical conductivity meter (Hi8633, Hanna Instrument, USA). After soaking of the seed at the stipulated soaking condition (temperature and time) with reversed osmosis water, all the steep water was poured into a beaker and stirred for 5 min before inserting the conductivity probe into the water. The conductivity reading was recorded when the reading was steady. Triplicate independent reading were recorded from the soaking solutions in the three beakers used for each soaking condition.

### 3.4.2 Swelling factor

The swelling factor of the seed powder suspension at 90 °C was determined using the methods of Tester and Morrison (1990) and Kaur and Sandhu (2010b). A sample weighing 100 mg was mixed with 10 ml of water in a 15 ml tared screw capped tube. The suspension was heated in a water bath at

90 °C for 30 min with a 5 min interval shaking in a vortex mixer. Once removed from the water bath, the suspension was cooled in ice and centrifuged at 3000 g for 10 min at 4 °C. The supernatant was discarded, and the weight of the sediment measured was found to be equivalent to the weight of swollen material. The swelling power was calculated with equation (5) as the ratio of the weight of the swollen material to the weight of the original dry sample.

$$\text{Swelling factor} = W_s / W_d \quad (5)$$

Where: $W_s$: weight of swollen sample, $W_d$ weight of original dry material

### 3.4.3 Water absorption index and water solubility index

The water absorption index (WAI) and water solubility index (WSI)of the seed flour and ground extrudate were determined at room temperature (20 °C) following the methods of Anderson (1982). A 100 mg powder or ground extrudate sample in a 15 ml tared screw capped tube was mixed with 5ml of water at room temperature (20 °C). The suspension was stirred intermittently with a vortex mixer for 3 h in the case of pigeon pea powder and for 30 min for the ground extrudate. The sample was centrifuged with centrifuge (Jouan CR31 multifunction, Thermo Electron corporation, Massachusetts, USA) at 3000 g for 10 min and at 4°C. The weight of the sediment was determined after decantation of the supernatant in a weighed aluminium dish.

The water absorption index was calculated with equation (6) as the ratio of the wet sediment to the dry sample. The water solubility index was determined after drying (section 3.2.3.4) for 16 h the supernatant in a weighed aluminium dish. The result was obtained through calculation with equation (7) as a percentage of the dry sample weight.

$$\text{Water absorption index (WAI)} = \frac{\text{weight of wet sediment}}{\text{weight of dry sample}} \quad (6)$$

$$\text{Water solubility index (WSI)} = \frac{\text{weight of dried solid in the supernatant x } 100}{\text{weight of dry sample}} \quad (7)$$

### 3.4.4 Texture analysis of seed and extrudate

The texture of the seeds was measured according to the method of Zamindar et al., (2013) while the extrudate texture was measured according to the method described by Ding et al (2006) and Moreira da silva (2014). The texture of the materials (seed or extrudate) was measured as the maximum peak deformation force. It is the maximum force required for the cylinder probe to penetrate the seed or extrudate and related to the sample hardness, hence imitating the force required for human mastication (chewing). The test was carried out with a Texture Analyser, (TA.XT Plus, Stable Micro System, Surrey UK) fixed with a 30 kg load cell and a cylindrical probe of 2 mm diameter. The cylindrical probe was programmed to approach either the seed or extrudate at 1 mm/s and on detection of the materials' surface, penetrated 60% of the cross-sectional diameter (cross sectional diameter was set at 2 mm for less expanded products and raw seeds and another 3 mm was used for highly expanded extrudates and cooked seeds) at 1 mm/s (test speed). Finally, the cylindrical probe returned to its initial position at 10 mm/sec (post speed). The degree of deformation and fracture of the material was shown in the test profile curve as force (N) distance (mm) curve and it represents the deformation force.

### 3.4.5 Extrudate expansion properties

#### 3.4.5.1 Extrudate bulk density

The bulk density of extrudate was determined by method of Lazou and Krokida (2010) using equation (8). The density was calculated as mass (g) per unit volume. Assuming the extrudate with cylindrical shape and volume as $(\pi d^2 l/4)$, the diameter (d) and length (l)were measured with digital

vernier caliper (Mitutoyo, Japan) while its mass was measured with an analytical balance. An average of 10 replicates was measured.

Bulk density ($g/cm^3$) $= 4m/(\pi d^2 l)$ (8)

### 3.4.5.2 Extrudate sectional expansion index (SEI)

The sectional expansion index (SEI) of the extrudate was obtained with the method of Alvarez (1988) and Lazou and Krokida (2010) by dividing the cross-sectional diameter of the extrudate with the diameter of the die. The cross-sectional diameter was determined as the average of 10 replicate measurement of a roughly 10 cm extrudate with a digital vernier caliper (Mitutoyo, Japan). The results were obtained using equation (9).

$$\text{Sectional expansion index} = \frac{\textbf{cross sectional diameter of extrudate}}{\textbf{diameter of die}} \quad (9)$$

### 3.4.5.3 Extrudate specific mechanical energy

The specific mechanical energy (SME) of the extrudate was calculated using the equation (10) described by Lai and Kokini (1991). The SME is the energy provided by the motor drive to the extruding material in the extruder per unit mass.

$$\text{SME (Wh/kg)} = \frac{\text{screw speed (rad s}-1) * \text{torque (Nm)}}{\text{feedrate}\left(\frac{\text{kg}}{\text{h}}\right) * 2} \quad (10)$$

Screw speed in rad $^{s-1}$ = screw speed in rpm * $2\pi/60$

### 3.4.6 Nutritional composition of seed flour and extrudate

The nutritional composition which comprises determination of total starch, resistance starch, amylose, protein, protein digestibility, amino acid and fibre for the seed flour and extrudate were determined.

### 3.4.6.1 Total starch content

Total starch of the seed powder and ground extrudate was determined according to AOAC Method 996.11 and AACC Method 76-13.01 using total starch assay kit from Megazyme international, Ireland.

This method was based on the following principle: The hydrolysis of starch by thermostable $\alpha$-amylase into soluble branched and unbranched maltodextrins and maltodextrins hydrolysis to D-glucose by amyloglucosidase. The oxidation of D-Glucoseto D-gluconate with GOPOD (Glucose oxidase plus peroxidase and 4-aminoantipyrin) and the released one mole of hydrogen peroxide is quantitatively measured in a colourimetric reaction at the production of a quinoneimine dye.

During the determination of the total starch, 100 mg of the ground sample was weighed into glass test tube (16 X 120 mm). The tube was tapped to ensure that all sample material dropped to the bottom of the tube. Aqueous ethanol (80%) measuring 0.2 ml was added to the sample to aid dispersion, followed by the addition of 2 ml of dimethyl sulphoxide (DMSO) and stirring with the vortex mixer. The tube was placed in boiling water bath for 5 min. Thereafter, 3 ml of thermostable $\alpha$ amylase (activity at 3000 U/ml; pH 5 and 40 °C) was added and incubated in a boiling water for 6 min with 2 min interval stirring with the vortex mixer. The stirring prevented development of lumps and expelling of ethanol during the heating as starch is being gelatinised. After cooling the sample to 50 °C in a water bath, 0.1ml Amyloglucosidase (330 unit/ml) was added and the sample incubated at 50 °C for 30 min. After the incubation, the content was transferred with the aid of a funnel to a 100 ml volumetric flask and the sample volume made to 100 ml with RO water before centrifugation of an aliquot of the diluted sample at 3,000 g for 10 min. The centrifuged aliquot was further diluted to 10 ml with distilled water and 0.1 ml was transferred to a new glass test tube (16 x 100 mm) for addition of GOPOD.

GOPOD measuring 3 ml was added into a separate new glass tube containing sample, reagent buffer and D glucose standard (1 mg/ml). The solutions were incubated at 50 °C for 30 min. Development of pink colouration was relative to the amount of starch in the sample through the measured absorbance at 510 nm ($\Delta A.$) and calculation with equation (11)

$$\% \text{ starch} = \frac{\mathbf{\Delta A} \times \mathbf{F} \times \mathbf{100} \times \mathbf{1} \times \mathbf{162} \times \mathbf{2} \times \mathbf{D}}{\mathbf{0.1} \times \mathbf{1000} \times \mathbf{180}} \quad (11)$$

Where

F (factor for conversion of glucose concentration from absorbance to microgram) = $\frac{100\ \mu g\ \text{of D-glucose}}{\text{absorbance for } 100\ \mu g\ \text{of glucose}}$

D = further dilution of the incubation mixture (when required)

100 = sample volume

1/1000 = conversion from µg to mg

0.1 = aliquot of sample analysed

162/180 = adjustment from free D-glucose to anhydro D glucose

2 = dilution of sample solution on incubation with amyloglucosidase

### 3.4.6.2 Starch digestibility using resistant starch assay

The starch digestibility of the native pigeon pea powder and ground extrudate were determined using resistant starch assay kit from Megazyme international Ireland. The resistant starch (RS) was calculated after the analysis with equation (12) and (13).

The first step in the analysis includes the 16 h enzymatic hydrolysis of the non- resistant starch. A ground sample measuring 100 g ± 5 mg was weighed into a corning culture tube (16 X 125 mm). A 4 ml volume of the combined enzymes of pancreatic alpha amylase (10 mg/ml) and amyloglucosidase (AMG 3 U/ml) was added to hydrolyse the non-resistant starch in 37 °C shaking water bath for 16 h incubation. Before aligning the tubes horizontally in the direction of motion in the water bath, the tubes were tightly capped tube was vortexed.

Second step: The sample after 16 h incubation was treated with 4 ml of 99% v/v ethanol to precipitate the resistant starch. The precipitated resistant starch was further separated after centrifugation at 1500 g for 10 min (non-capped) while the non-resistant starch(supernatant) was

decanted into a tube for further use. The precipitated resistant starch (residue)was washed with 2 ml and 6 ml of 50% ethanol after which it was centrifuged at 1500 g for 10 min for separation of the ethanol rich supernatant. To ensure the resistant starch was free of ethanol, the tube was inverted to drain off ethanol.

Step three: The procedure for the determination of the resistant starch in the residue continued with the addition of two magnetic stir bars and 2 ml of 2 M KOH into the tube containing the resistant starch. This solution was stirred for 20 min in cold water ice placed on a magnetic stirrer. Constant stirring was applied to avoid formation of lumps. A volume of 8 ml of 1.2 M sodium acetate buffer and 0.1 ml of AMG (solution 1:3300 u/ml) were added into the tube and with vigorous stirring on a vortex mixer for 1 min. The sample was then incubated in a water bath at 50 °C for 30 min. Based on literature which reported that the resistant starch of native pigeon pea seed flour was more than 10% (Kasote et al., 2014), the content of the tube containing the native pigeon pea was diluted by adjusting the volume to 100 ml with reversed osmosis water. The solution was centrifuged at 1500 g for 10 min. Meanwhile, according to assay protocol and literature, the tubes containing the resistant starch from the extrudate were centrifuged directly at 1500 g for 10 min without dilution. Sample aliquot of 0.1 ml was transferred to 16 X 100 mm tube where 3 ml of GOPOD reagent was added. Furthermore, 3 ml of GOPOD was also added to the blank [0.1 ml of 100 mM sodium acetate buffer (pH 4.5)] and glucose standard (0.1 ml of mg/ml d-glucose). Following the incubation with GOPOD for 20 min at 50 °C, the absorbance of the samples was measured at 510 nm against the reagent blank.

Step four: This step involves the determination of non-resistant starch. Following the washing of non-resistant starch (supernatant) from the 16 h incubated with 50% ethanol, the volume was adjusted to 100 ml with sodium acetate (pH 4.5). From this diluted solution, an aliquot of 0.1 ml of

the non-resistant sample plus 10ul of dilute AMG solution (300 U/ml) was incubated for 20 min at 50 °C. After this incubation, 3 ml of GOPOD was added to all the samples and another incubation at 50 °C for 20 min was carried out. The absorbance was then measured at 510 nm after the incubation with the GOPOD.

Finally, the resistant and non-resistant starch content of the samples were calculated from the equations 12-14 while the starch digestibility was obtained from equation 16 by calculation of the percentage of non-resistant starch in the material over the total starch content.

**Calculations**:

Resistant Starch (g/100 g sample) for samples containing > 10%RS

$= \Delta E \times F \times 100/0.1 \times 1/1000 \times 100/W \times 162/180$

$= \Delta E \times F/W \times 90$ (12)

Resistant Starch (g/100 g sample) for samples containing < 10% RS

$= \Delta E \times F \times 10.3/0.1 \times 1/1000 \times 100/W \times 162/180$

$= \Delta E \times F/W \times 9.27$ (13)

Non-Resistant (Solubilised) Starch (g/100 g sample)

$= \Delta E \times F \times 100/0.1 \times 1/1000 \times 100/W \times 162/180$

$= \Delta E \times F/W \times 90$ (14)

Total starch = Resistant starch + Non-resistant starch (15)

Starch digestibility (%) = non-resistant starch/total starch *100 (16)

Where:

ΔE = absorbance read against the reagent blank.

F = conversion from absorbance to micrograms calculated with the absorbance obtained for 100 µg of D-glucose in the GOPOD reaction

= 100 (µg of D-glucose) divided by the GOPOD absorbance for this 100 µg of D-glucose.

100/0.1 = volume correction (0.1 ml taken from 100 ml).

1/1000 = conversion from micrograms to milligrams.

W = dry weight of sample analysed = weight x [(100-moisture content)/100].

100/W = factor to present RS as a percentage of sample weight.

162/180 = factor to convert from free D-glucose, as determined, to anhydro-D-glucose as occurs in starch.

10.3/0.1 = volume correction (0.1 ml taken from 10.3 ml) for samples containing 0-10% RS and where the solution incubated at 50 °C was not diluted and the final volume is ~ 10.3 ml.

#### 3.4.6.3 Protein content with BCA protein assay

The protein assay was carried out with the Pierce BCA Protein Assay Kit. This method according to the manual, combines the reduction of $Cu^{+2}$ to $Cu^{+1}$ by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation ($Cu^{+1}$) using a unique reagent containing bicinchoninic acid. The purple-coloured reaction product (water-soluble complex) of this assay which is formed by the chelation of two molecules of BCA with one cuprous ion exhibits a strong absorbance at 562 nm. The absorbance (colour change) is nearly linear with increasing protein concentrations over a broad working range (20-2,000 µg/ml). The BCA method is not a true end-point method; that is, the final colour continues to develop. However, following incubation, the rate of continued colour development is sufficiently slow to allow large numbers of samples to be assayed together.

##### 3.4.6.3.1 Sample preparation for BCA protein assay

Prior to the determination of the protein content with BCA reagent, the extraction of soluble protein from 0.1 g of powdered sample with 1 ml of

2% SDS solution (prepared mixing 2 g of SDS in 98 ml of pure water) was carried out. The sample suspension was heated in a water bath at 60 °C for 30 min. Following this, the sample was vortexed for 1 min and subsequently centrifuged at 13,000 rpm for 3 min. The sample was re-heated in the same water bath at 60 °C for 30 min after which it was diluted to 1/100 in 2% SDS solution (0.1 ml of sample + 9.9 ml of 2% SDS solution). A working reagent was prepared from 50 parts of reagent A (sodium bicarbonate, bincinchonic acid and sodium hydroxide) and 2 parts of reagent B (cupric sulfate).Before 30 min incubation at 37 °C, 2 ml of the standard working was added into 100 ul of diluted sample measured in triplicates and the protein standards prepared from bovine serum albumin according to section 3.4.6.3.2. The absorbance of cooled samples, standards and blank were measured with a spectrophotometer (LKB Biochrom Ultrospec 450) set at 562 nm. The absorbance of all the standards and sample replicates were subtracted from the blank followed by plotting a standard curve with standards absorbance against concentration in µg/ml. The samples protein concentrations (µg/ml) were read from the standard curve while final protein content in % dwb was calculated using the equations in section 3.4.6.3.3.

#### 3.4.6.3.2 Preparation of standard curve:

Different sets of protein standards were prepared by diluting the content of one albumin standard (Bovine Serum Albumin) ampule into several standards in clean vials using the same diluent as the sample (2% SDS solution). According to Table 3-7, standards were pipetted into a labelled test tube followed by the addition of 2.0 ml of the standard working reagent. The protein standard solutions in the vials were vortexed and incubated at 37 °C for 30 min together with the samples prepared in section 3.4.6.3.1.

**Table 3-7:** Dilution scheme for protein standard according to Pierce BCA protein assay test tube protocol. (Protein standard working range = 20–2,000 µg/ml).

| Vial | Volume of Diluent | Volume and Source of BSA | Final BSA Concentration |
|---|---|---|---|
| A | 0 | 300 µl of Stock | 2,000 µg/ml |
| B | 125 µl | 375 µl of Stock | 1,500 µg/ml |
| C | 325 µl | 325 µl of Stock | 1,000 µg/ml |
| D | 175 µl | 175 µl of vial B dilution | 750 µg/ml |
| E | 325 µl | 325 µl of vial C dilution | 500 µg/ml |
| F | 325 µl | 325 µl of vial E dilution | 250 µg/ml |
| G | 325 µl | 325 µl of vial F dilution | 125 µg/ml |
| H | 400 µl | 100 µl of vial G dilution | 25 µg/ml |
| I | 400 µl | 0 | 0 µg/ml = Blank |

#### 3.4.6.3.3 Equations for Calculation of protein content from standard curve

(1) % Protein content = dilute sample protein concentration (µg/ml) from standard curve /original sample concentration (µg/ml) x 100

(2) Original sample concentration (g/ml)=original weight of sample (g) / volume of diluent (ml)

(3) Volume of diluent = volume of 2% SDS used in dilution

Original sample concentration in g/ml was changed (to µg/ml=g/ml *1000000) before the final calculation of protein content

(4) Protein in % dry weight basis = 100%* protein on wet basis /dry matter (DM)

(5) dry matter (DM) = 100- moisture content

### 3.4.6.4 Protein content of the extrudate by Dumas combustion method (1826)

The crude protein content of the extrudates was determined by the Dumas combustion method using an Organic Elemental Analyser (Flash 2000 Organic Elemental Analyser, Thermo Scientific, Italy). This method was used to determine the protein content of the ground extrudates as it was more automated for routine analysis without the use of chemical reagents.

The principle of the method is based on the liberation of nitrogen (with other elements such as carbon, sulphur and hydrogen) from the breaking of biopolymers in a short time (flash time) with high combustion temperature (900 °C). Following the liberation of nitrogen and detection by the thermo conductivity detector attached to the gas chromatography column, the total nitrogen was measured. The percentage protein content was calculated by multiplying the total nitrogen by a factor 6.25.

**Procedure**

The extrudate was weighed (5-6 mg) in an aluminium tin capsule and then folded before inserting of the capsule into the Organic Elemental Analyser auto sampler. Similarly, the 2-3 mg of the standard (Sulphanilamide) was weighed into the capsule and then inserted into the auto sampler after having been folded. The samples were combusted with oxygen in the 900 °C furnace of the Organic Elemental Analyser for a few seconds (flash time) and the nitrogen content from the chromatograph was converted to crude protein content.

### 3.4.6.5 Protein digestibility

The *in vitro* protein digestibility (IVPD) of the extrudate was carried with multi-enzyme technique according to Hsu et al. (1977) reported by Khattab et al. (2009). The principle involves the digestion of protein with the three principle enzymes used *in vivo* digestion of protein. The quantification of the protein hydrolysis was noted as a change in pH due to the release of amino acid at a fixed time interval. Powdered sample weighting 1 g was dissolved (solubilised) in 50 ml of distilled water and hydrated for 1h to extract protein (6.25 mg protein/ml) from sample suspension. The pH of the sample suspension was adjusted to 8 using 0.01 mol KOH. The suspension was incubated in a shaking water bath at 37 ºC for 30 min. The fresh multi-enzyme solutions at pH 8 was prepared to contain 1.6 mg trypsin (23,100 U/ml), 3.1 mg chymotrypsin (186 U/ml) and 1.4 mg

peptidase (0.952 U/ml) dissolved in 1ml distilled water. Five ml of the enzyme solution was added to each sample suspension with constant stirring at 37 ºC for 15 min. The pH of the sample suspension was recorded after the 15 min of addition of enzyme solution and stirring. The *in vitro* protein digestibility (IVPD) was calculated with equation (17)

$$Y = 210.464 - 18.103x \quad (16)$$

Where

y =IVPD (%)

X=pH of the sample suspension after 15 min.

### 3.4.7 Amino acid protocol

#### 3.4.7.1 Principle

The amino acid composition was determined utilising the oxidative – hydrolysis method. The principle was as follows: the sample was oxidised with a hydrogen peroxide/formic acid/phenol mixture. Excess oxidation reagent was decomposed with sodium metabisulphite. The oxidised sample was hydrolysed with 6 M hydrochloric acid for 24 h. The hydrolysate containing the amino acid was adjusted to pH 2.2, centrifuged and filtered. The amino acids were separated by ion exchange chromatography and determined by the reaction with ninhydrin using photometric detection at 570 nm (440 nm for proline) and finally calculated with equation (18).

#### 3.4.7.2 Preparation of reagents used in amino acid protocol

Before the analysis, certain reagents were prepared as follows: formic acid/phenol solution was prepared by adding 735 ml of formic acid to 111 ml of deionised water together with 4.73 g of phenol in the fume cupboard with a face mask. Hydrolysis reagent was prepared by adding 492 ml of HCl and 1 g of phenol to 300 ml deionised water and solution made up to 1 L with the deionised water.

**Preparation of amino acid standard solutions**

Oxidation solution was prepared 2 h prior to oxidation of samples by dispensing 10 ml of 30% hydrogen peroxide into a 100 ml volumetric flask and the volume made up with formic acid/phenol solution. The solution was incubated at 30 °C for 1 h and was observed to turn a yellow colour. It was cooled in a fridge before adding to the samples. The 25% of 2,2'-thiodiethanol solution was prepared by measuring 50 ml of 2,2'-thiodiethanol into a 200 ml volumetric flask. The volume was made up to 200 ml with deionised water. Sodium citrate (150 mM) buffer pH 2.2 was prepared by mixing 39.22 g of tri-sodium citrate with 20 ml of 25%-2, 2'-thiodiethanol solution, 2 g of phenol and 33 ml of hydrochloric acid in 1400 ml of deionised water. The pH of the oxidation regent was adjusted to 2.20 using 7.5 N sodium hydroxide.

The amino acid standard solutions were prepared as follows: concentrated solution of cysteic acid and methionine sulfone (1.25 mol/ml) was prepared by dissolving 0.2340 g of cysteic acid and 0.2265 g of methionine sulfone in 200 ml pH 2.20 tri-sodium citrate buffer (or Loading Buffer) in a 1 l volumetric flask and volume made up to 1 l with pH 2.20 tri-sodium citrate buffer. Concentrated solution of the internal standard norleucine (10 mol/ml) was prepared by dissolving 0.6560 g of norleucine in 100 ml pH 2.20 tri-sodium citrate buffer in a 500 ml volumetric flask and volume made up to 500 ml with pH 2.20 tri-sodium citrate buffer. A working standard solution was made by the addition of 80 µl of 200 nmol/ml (µM) amino acid standards (18 amino acids), 80 µl of 100 nmol/ml (µM) concentrated solution of cysteic acid and methionine sulfone and 20 µl of 200 nmol/mL (µM) of concentrated internal standard norleucine to a HPLC vial including 820 µl sodium citrate buffer.

### 3.4.7.3 Protocols for determination of amino acid

Ground extrudate samples and native pigeon pea weighing 500 mg (containing approximately 10 mg nitrogen) was transferred into a 100 ml sample bottle. The sample bottle was placed in a fridge to cool followed by

addition of 5 ml of chilled oxidation solution. Afterwards, sample bottles were returned to the fridge for overnight oxidation (a minimum of 16 h) of the sample.

After oxidation, the samples were removed from fridge and 0.84 g of sodium metabisulphite was added to each. This decomposed any excess oxidation reagent. Furthermore, after addition of 50 ml of the hydrolysis reagent (6 M HCl) to the sample, the bottle lids were loosened to prevent a build-up of gas pressure and then placed back in an oven at 110 °C. At the end of 1 h hydrolysis, the sample bottle lids were tightened wearing protective clothing and then left in the oven at 110 °C for further 23 h hydrolysis.

After hydrolysis, the sample was removed and place in a freezer for 45 min. Following removal of the samples from the freezer, it was transferred into 250 ml wide necked conical flasks and the initial sample bottle washed with pH 2.2 tri-sodium citrate buffer. The pH of the neutralising regent (7.5 N sodium hydroxide) was adjusted to 2.2 using 1 N sodium hydroxide and hydrolysis reagent before neutralising the sample at room temperature for 30 min. with 35 ml of the neutralising regent.

The neutralised hydrolysate at pH 2.2 was transferred to 200 ml volumetric flasks to which 4 ml of internal standard norleucine was added. The sample bottle was rinsed using tri-sodium citrate pH 2.2 following which the flask volume was filled to 200 ml. Furthermore, 20 ml of diluted hydrolysate was transferred to a centrifuge tube and centrifuged at 3000 g for 2 min. The supernatant was filtered with a 0.22 µm filter stringe into a sterile sample vial and loaded onto the amino acid analyser (Biochrom 20+, Biochrom Ltd Cambridge) for amino acid compositional analysis

The concentration of amino acid was expressed as gram per kilogram sample (g/kg or mg/g) and calculated as follows:

Amino acid per kilogram sample (g/kg) = (A*MW*F)/ (W*50000) (17)

A = concentration of hydrolysate obtained from the amino acid analyser instrument (ISTD-nmol / 50 µl)

MW = molecular weight of amino acid

E = concentration of standard in µmol/ml (or nmol/µl)
W = g sample (corrected to original wt if dried or defatted)
F = ml total hydrolysate

Cystine and cysteine were both determined as cysteic acid in hydrolysates of oxidised samples but calculated as cystine by using MW 240.3. Methionine was determined as methionine sulfone in oxidised samples but calculated as methionine by using MW 149.21.

### 3.4.8 Determination of lipid content using Bligh and Dyer Method (1959)

The principle of the Bligh and Dyer (1959) method was based on the multiple extraction and purification of the lipid from a wet mixture of biological material with a mixture of chloroform and methanol and formation of a homogenous miscible system. The dilution with chloroform and water enables the separation of lipid in the chloroform layer (bottom phase) and non-lipid component in the methanol layer.

**Procedure**

A known weight (0.1 g) of milled pigeon pea seed powder was weighed into a 15 ml centrifuge tube (A) and mixed with 0.4 µl water to form a wet mixture. The wet mixture was vortexed in a roller mixer for 1 min. A homogenized mixture (homogenous miscible system) of chloroform–methanol (2:1) was prepared by mixing 10 ml of chloroform with 5 ml of methanol. To form the homogenised mixture with the sample, 1 ml of the chloroform-methanol mixture was added to the wet sample and the new mixture vortexed for 1 min. The homogenate was centrifuged at 3000 g for 5 min at 4 °C. From the bottom phase (chloroform phase), 500 µl of the solution was transferred into a 2 ml centrifuge tube (tube B). The second extraction and third extraction were completed by the addition of another 500 µl of chloroform- methanol mixture to tube (A) and repeating the process. The whole extract in tube (B) was mixed well and then vortexed.

From the extract in tube (B), an aliquot of 1 ml was transferred into a weighed 2 ml glass bottle. The aliquot in the glass bottle was dried in the vacuum oven at 40 °C for 14 h. The dried lipid was cooled in a desiccator followed by weighing of the dried lipids. The lipid content was calculated using equation (18)

$$\%\ \text{lipid} = \frac{\text{wt of lipid} * \text{vol of chloroform} - \text{methanol used}}{\text{wt of sample} * \text{vol of extract aliquot}} * 100\% \qquad (18)$$

Where: Vol of chloroform- methanol=2000 µl
Vol of extract aliquot = 1 ml=1000 µl

### 3.4.9 Dietary fibre

The dietary fibre was determined using the enzymatic-gravimetric method according to AOAC 985.21 and 991.43 methods. The procedure involved digestion of starch with heat stable $\alpha$ amylase for hydrolysis of soluble unbranched starch, and gelatinisation, followed by enzymatic digestion with protease and amyloglucosidase to remove protein and starch respectively. Part of the procedure was the addition of ethanol to facilitate the separation of soluble fibre from the insoluble fibre. The% dietary fibre was calculated with equation (20) after subtraction of the weight of residual protein and ash from the residual weight.

The seed flour or extrudate (1 g) was weighed into each of eight beakers. Four of the sample beakers were designated for either total or insoluble dietary fibre. Phosphate pH 6 buffer measuring 50 ml was added, followed by addition of 0.1 ml heat stable $\alpha$ amylase and gelatinisation at 95 °C for 30 min. After cooling the sample, the pH was adjusted to 7.5 with 10 ml of 0.275 N NaOH. The protein was digested with 0.1 ml of pronase enzyme (50 mg/ml) at 60 °C for 30 min in a water bath. However, after cooling, 10 ml of 0.325 M HCl was used to re-adjust the pH to 4 followed by addition of 0.1 ml amyloglucosidase for starch digestion through incubation at 60 °C for 30 min. During the incubation with enzymes, the beakers were covered with aluminium foil.

After the incubation with amyloglucosidase, 4 volumes of 95% ethanol (by weight of the sample) was added to the sample to allow for the overnight precipitation of total fibre, whereas none of 95% ethanol was added to the beakers for insoluble fibre. All the samples were filtered through a weighed fritted crucible of porosity 2 (40-60μm pore size). After filtration, the residue was washed with 60 ml of 68% ethanol, followed by 20 ml of 95% ethanol and lastly with 20 ml acetone.

To calculate the % dietary fibre with equation (19), the separate weight of the residue in the crucible from the total dietary fibre (ethanol precipitate) and insoluble fibre were measured after drying with forced air drier at 105 °C. The average weight of ash and protein in the residue were also determined and subtracted from the dried residue weight to give % dietary fibre.

$$\%\ \text{dietary fibre} = \frac{(\text{weight of dried residue} - \text{protein} - \text{ash}) * 100}{\text{sample weight}} \quad (19)$$

## 3.5 Anti-nutritional factors

### 3.5.1 External laboratories anti-nutritional factor analysis

Due to the high risk involved in the analysis, the anti-nutritional factors listed in Table 7 were analysed by external laboratories.

**Table 3-8:** Anti-nutritional analysis by external laboratories.

| Anti-nutritional compound | Method | Name of External Lab |
|---|---|---|
| Lectin | Elisa | Triskelion,Utrechtseweg 48, 3704 HE Zeist, Netherlands |
| Alkaloid | VDLUFA M16.2.1 | Triskelion, Utrechtseweg 48, 3704 HE Zeist, Netherlands |
| Cyanide | EC 71/250- | Triskelion, Utrechtseweg 48, 3704 HE Zeist, Netherlands |
| Trypsin inhibitor | AACC22-40-01 | Medallion Lab,9000 Plymouth Ave N, Minneapolis, MN 55427, United States |
| Oxalate | Organic acid AOAC 986.13 | Medallion Lab,9000 Plymouth Ave N, Minneapolis, MN 55427, United States |
| Phytic acid | K-PHYT 12/2-Megazyme (Wicklow,Ireland) | Medallion Lab,9000 Plymouth Ave N, Minneapolis, MN 55427, United States |

## 3.6 Extraction of starch

Starch was extracted from pigeon pea using the wet milling method of Hoover *et al* (2010). The extracted starch was used for evaluation of thermal behaviour and microscopy of pigeon pea. Pigeon pea weighing 500 g was soaked with 2 L of reversed osmosis water for 6 h. The suspension was wet milled with a Kenwood blender after decantation of the soaking water. The wet milled suspension was filtered through a muslin cloth. The filtrate was allowed to sediment for 2 h and supernatant discarded. Reversed osmosis water measuring 500 ml was added to the filtrates and pH adjusted to 9 with 1M NaOH (pH of the 1M NaOH=13.11) to remove protein.

The adjusted starch solution after 2 h was centrifuged at 3000 g for 5 min at 4 °C with a Beckman J2-21M induction drive centrifuge. The suspension was neutralised to pH 7 with 0.1N HCl (pH of 0.1N HCl=2.27) and centrifuged at 3000 g, 4 °C for 5 min. The sediment which is the extracted starch was freeze dried and stored at 4 °C for further analysis.

## 3.7 Thermal behaviour

The thermal behaviour of pigeon pea seed powder, starch and extrudate in high and low moisture-heat environment were assessed with a Rapid Visco Analyser (RVA) and a Differential Scanning Calorimeter (DSC) respectively. The thermal behaviour was related to the starch gelatinisation characteristic.

### 3.7.1 Pasting profile and properties

The pasting properties of treated and non-treated seed flour were determined using the Rapid Visco Analyser (Super 4, Newport Scientific Ltd, Australia). The Rapid Visco Analyser (RVA) is widely used to measure stirred viscosity of starch or starchy material suspensions subjected to

time/ temperature protocols. The viscosity also shows starch integrity and swelling potentials of the material. The viscosity is measured as the torque required for the stirring of the paddle. The full details of the RVA pasting curve is in Figure 3-3 and section 3.7.1.1.The viscosity measured gives an indication of gelatinisation behaviour of material in excess water.

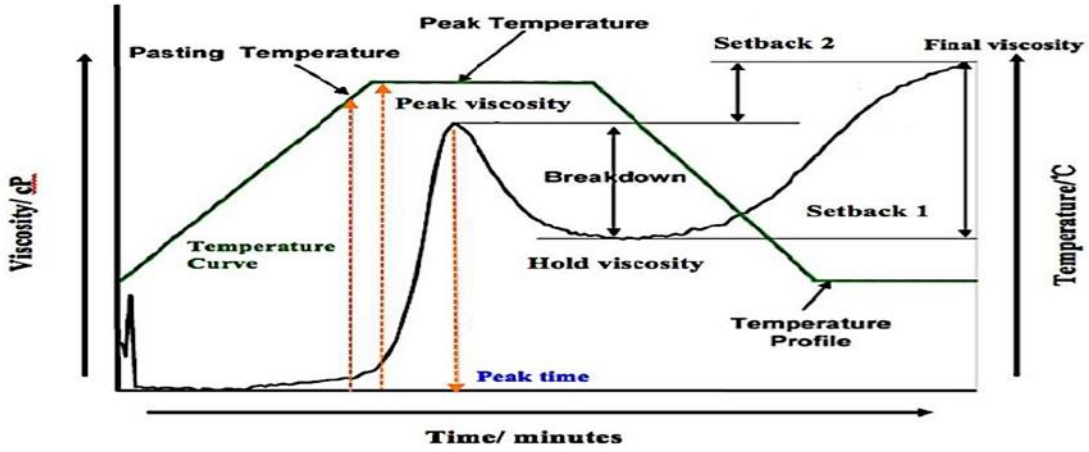



**Figure 3-3:** Prototype of RVA pasting curve.

### 3.7.1.1 Important features of the RVA pasting curve according to the equipment manual

**Pasting temperature**: The temperature at the on-set of rise in viscosity. It provides an indication of the minimum temperature required to cook a given sample. The high value indicates the resistance of the granule to swelling.

**Peak viscosity**: Highest viscosity achieved by the sample at the beginning of heating at the profile highest temperature which is usually between the swelling of starch granule and polymer leaching. It also indicates the water binding capacity of the mixture. The higher the value, the more the presence of gelatinised starch granule in the paste.

**Peak time**: The time at the on-set of rise in viscosity. The lower the time, the ease in the formation of the peak viscosity.

**Trough**: The minimum viscosity at holding period when heating is at the highest temperature of the profile. Generally, occurs after the peak viscosity.

**Breakdown**: The difference between peak viscosity and trough. Low breakdown indicates the stability of the hot paste during stirring and heating cycle.

**Setback viscosity**: The phase which involves re-association of starch molecules. It is calculated as the difference between the peak and final viscosity. Depending on the loss of kinetics during cooling, re-association will occur. The higher the value the re-association, the higher the set back on the cold paste.

**Final viscosity**: Sample viscosity at the end of the pasting analysis at the lowest cold temperature profile or alternatively called cold paste viscosity. It defines samples quality and indicate the ability of the sample to form a gel after cooling.

**Cold water viscosity**: The viscosity at the lowest starting temperature of the profile.

#### 3.7.1.2 Native seed flour pasting properties

The 'Standard 1' profile was used during the pasting analysis. This consists of a starting temperature of 50 °C which is kept constant for 1 min before it is raised to 95 °C in 3.5 min, kept constant at 95 °C for 3 min, decreased to 50 °C in 3.5 min and finally kept at this temperature for 2 min.

Flour samples for RVA analysis were prepared in the aluminium canisters with 12.5% solids content (dry basis) and made up to 28 g with deionised water. A stirring paddle was inserted into the canister (to stir the powdered suspension at a steady shear rate) before mounting it into the RVA sample holder. Each sample was prepared and analysed in triplicate and the results of pasting viscosities (peak, breakdown, setback and final), peak temperature and peak time were reported as mean plus standard deviation.

### 3.7.2 Extrudate pasting properties

The pasting characteristics of the extrudates were determined using the same equipment (Rapid Visco Analyser super 4 models by Newport

Scientific Ltd, Australia) as in section 3.7.1 and RVA curve Figure 3-3. The protocol was modified from Carvalho and Mitchell (2001) to detect cold water viscosity. The profile applied consisted of a starting temperature of 25 °C which was kept constant for 5 min before it was raised to 95 °C in 4min, kept constant at 95 °C for 2 min, decreased to 25 °C in 4 min and finally kept at this temperature for 5min.

Ground extrudate 5 g (dry basis) was weighed into canisters and was solubilised with 2 ml-100% absolute ethanol to prevent lump formation. The mixture was made up to 28 g with deionised water. Each sample was prepared and analysed in triplicate and the results of cold water viscosity was determined at 5 min of the starting time followed by the peak viscosity at 10 min of the pasting period. All values were mean plus standard deviation.

The measured cold water peak viscosity of the extrudate is an indication of starch gelatinisation during the extrusion processing. It is expected that the cold water viscosity of the extrudates will increase if the degree of cook (gelatinisation) during the extrusion increases and then decreases at higher pasting temperature due to granular rupture and dextrinization. However, if the extrudate final viscosity from the pasting profile is higher, it will indicate that lower gelatinisation of starch occurred during the extrusion.

### 3.7.3 Thermal properties of pigeon seed flour, starch and extrudates using Differential Scanning Calorimeter (DSC)

The thermal properties of pigeon pea powder, starch and extrudates were determined using the Differential Scanning Calorimeter (DSC823, Mettler Toledo, Switzerland) which measures the gelatinisation temperature ranges and heat capacity. The wet-milled samples were freeze dried before the calorimetric measurement. The samples were weighed (3 mg) into 120 ml stainless steel pan and water measuring three times (9 µl) of the sample weight was added. The pans were hermetically sealed and left for equilibration at room temperature for 24 h.

The samples after equilibration were placed in the DSC auto sampler and heated from 20-120 °C at a heating rate of 10 °C/min. The thermal properties which include the gelatinisation enthalpy and temperatures of onset, peak and end-set gelatinisation were depicted under the area of the DSC profile curve. Software was used for the conversion of raw data to the gelatinisation properties.

### 3.7.4 DSC thermal properties of pigeon pea protein isolate

The protein was first isolated from the pigeon pea seed prior to the determination of the DSC thermal properties using the Differential Scanning Calorimeter (DSC823, Mettler Toledo, Swizerland). The isolation technique described by Paredes-Lopez and Ordorica-Falomir (1986) and reported by Mwasaru *et al*. (1999) was applied with some modifications. The thermal behaviour of the isolated protein will enable proper assessment of the contribution of protein to the overall thermal behaviour of pigeon pea.

During the extraction of protein, 30 g of pigeon flour was mixed with 300 ml of 0.1 M NaOH (1:10 flour: solvent, w/v). Before the extraction, the pH of 0.1 M NaOH was adjusted to 8.5 with sodium phosphate buffer of pH 6. The extraction was carried out for 12 h (overnight) on a roller mixer (Stuart, UK). The suspension was centrifuged (Beckman J2-21M induction drive centrifuge) at 5000 g for 15 min at 10 °C. The supernatant was decanted, and the residue filtered with grade 2-270 mm circle whatman filter paper.

The protein was further precipitated by adjusting the pH to 4.5 using 0.1 M HCl at ratio of 10:3 (supernatant: acid). The solution was observed to turn milky after the pH adjustment. The precipitate was allowed to form and settle for 2 h. The precipitated protein was recovered by centrifuging at 5000 g for 10 min @ 10°C, followed by washing three times in excess water and centrifugation. The isolate was freeze dried.

The DSC thermal properties were determined according to section3.7.3

## 3.8 Structural characteristics

### 3.8.1 X-ray diffraction pattern and relative crystallinity

The X-ray diffraction analysis was applied to evaluate the crystalline structure of the samples. The X-ray diffraction pattern and relative crystallinity value of starch, native and treated flours were determined with a wide angle X-ray powder diffractometer (D5005, Siemens AG, Germany) with copper target and Alpha emission (Cu Kα) at 40 mA, 40 KV. The diffractograms were obtained from 3-38 thetas (2θ), spinner stage rotation at 60 rpm, and scan rate of 0.05 º (Angle degree step) per 2.5 sec for 29 min. The relative crystallinity defined as the total crystalline region/portion based on the size and quantity of crystallites was calculated through the system software by assigning the upper diffraction peak area that comprises the sharp peak as crystalline portion, while the lower broad area was assigned the amorphous peak. The baseline used was from 5-37 $^{0}$theta.

### 3.8.2 Scanning electron microscopy

The morphological changes in the pre-treated seeds, seed flour and extrudate were examined with a scanning electron microscope (SEM) (JSM-6490LV JEOL., USA) The SEM was carried by Christine Grainger-Boultbya Technician at Boot Science Building, School of Pharmacy, The Scanning electron microscope was operated at 15 KV. The wet milled samples were freeze dried before the microscopic examination while dry milled samples were used unaltered. The samples were embedded in the stub before the gold plating. The gold plated samples were then examined for its microstructure using the Scanning electron microscope.

### 3.8.3 Fluorescence microscopy

Fluorescence microscopy was used to characterise extrudate microstructure and to visualise the impact the interactions between the

major feed chemical components (starch, protein and dietary fibre which consists of seed coat and cell wall materials) had on the extrudate structural integrity. The epifluorescence microscope (DMRB, Leica Microsystems Wetzlar, Germany) with a wide field fluorescence microscope was used during this investigation. The application of the fluorescence dyes (FTIC, rhodamine B and calcofluor white) to the samples enabled the samples to become fluorescent. The extrudates were prepared for microscopic examination using these following procedures. The Technician (Ian Ward) in E100 Lab QMC assisted in the imaging.

#### 3.8.3.1 Preparation of sample blocks (moulding)

The extrudates measuring 2 cm were soaked with 50 ml of water in 100 ml beaker for 30 minutes. During the soaking period, the aluminium foil mould required for making the sample block was formed. To make the mould, aluminium foil was wrapped around the outside of a 10ml sample bottle. The mould was then inserted into dry ice to condition the temperature. At the end of the 30 minutes soaking of the samples, the wet sample was inserted into the middle of the aluminium mould followed by the pouring of optimum cutting temperature (OCT) mounting medium for cryotomy over the specimen. The sample was then allowed to set in the OCT until it was frosty, usually for 30 min to form the sample block. After the sample had become frozen, the mould was dismantled by peeling off the aluminium to enable the sample block to emerge. The sample block was packed in labelled bags and stored in the cryostat set at -20 °C for immediate sectioning.

#### 3.8.3.2 The mounting, sectioning of samples with cryostat and fixation of sample cell in the slide

Following the lubrication of the surface of the chucks with some OCT, the specimen was mounted on the chucks for 1 min before inserting the chucks into the chucks holder in the cryostat for sectioning.

The sectioning (cryotomy) of the specimen block was performed with a cryostat microtome (CM 3505-S, Leica Microsystems Wetzlar, Germany) The cryostat temperatures for sectioning the specimen were set at -21 °C for the chamber temperature and -19 °C for the object temperature. The thickness of the sectioned specimen was set at 10 µm. Following the sectioning, the sectioned specimen was embedded on an already prepared gelatinised slide (gelatine coated slide) and stored in the cryostat for staining.

#### 3.8.3.3 Staining protocol

The specimen in the slide was adjusted to room temperature followed by marking the area covered by the specimen with an Immedge pen (Hydrophobic barrier pen). The slides for the staining were placed in a humidifying area. Multi- staining of the specimen with 50 µl of each of the three fluorescence dyes (calcofluor white, FITC and Rodamine B) was carried out at 5 minutes intervals. Fluorescent mounting medium measuring 6 µl was added to the surface of 0.17 mm thick cover slip before using the stained cover slip side to cover the specimen slide. Following the covering of the specimen, the boundaries between the coverslip and the specimen was sealed with nail polish. The stained specimen slides were then stored in a refrigerator ready for observation with the fluorescence microscope.

#### 3.8.3.4 The viewing of the extrudate cellular components

Before the viewing of the specimen cell, the excitation and emission filters (wavelength) were selected according to the specification for the different dye used.

**Table 3-9:** Optical filters used in viewing the fluorescence images.

| Extrudate cellular components | Channel (light part) | Excitation filter(nm) | Emission filter(nm) |
|---|---|---|---|
| Fibre (Cell wall materials) | DAP (blue) | 402/15x | 455/50 |
| Starch | FITC (green) | 490/15x | 525/36 |
| Protein | McCherry (red) | 577/25 | Multi–band emission |

## 3.9 Statistical method

The significant difference ($P<0.05$) of the mean of all the data generated during this study was assessed using two-way Anova (multivariate) under General linear model of IBM SPSS statistics 24 software. Following the analysis of variance, the mean values were compared and separated into letter (alphabets) subscripts using Tukey *post Hoc* test.

# 4 Physicochemical characteristics of three varieties of pigeon pea

## 4.1 Introduction

In this chapter, the result of the physico-chemical characteristics (hydration capacity, chemical composition and pasting properties) of three commercial varieties of pigeon pea, namely white Agatu, red Agatu and Bayelsa are reported and discussed. The physico-chemical characteristics were studied to establish if there were differences between the samples significant enough to warrant specific choice of variety for future work. The three varieties which were native to Nigeria were initially selected based on their easy cultivation, yield after harvest and commercial availability. Among the varieties, local consumers typically prefer white Agatu because it is cheaper, easy to cultivate and provides a high yield.

Before preparing conventional foods based on pigeon pea, the seed requires hydration and this is achieved by soaking the seed. After reporting some preliminary results on seed hydration and physical properties, the results given and discussed in this chapter were all obtained based on the conventional soaking method section 3.2.1. This conventional soaking method uses four times as much water, by weight in relation to the seeds and the duration of soaking was for 12 hours at ambient temperature (20 °C). After soaking, some seeds were dehulled (meaning the seed coat was removed). The reason for this is because data shows consumers' preference to be for products made from dehulled (removed seed coat) seed flour (Igbedioh et al., 1994, Fasoyiro et al., 2010, Tiwari et al., 2010, Akinjayeju and Ajayi, 2011). Both the soaked seeds and dehulled seeds were dried in a convection oven at 50 °C for 12 h while the milled native seed served as the control. All these samples were used for the determination of the chemical composition and the pasting properties of the three pigeon pea varieties.

## 4.2 Indication of hydration of pigeon pea seed

Preliminary work was undertaken to investigate the impact of hydration of the pigeon pea. The seeds were soaked at 50 °C for a short period (30-60 min) to establish the hydration pattern. The pigeon pea is a relatively small legume seed (Figure 4-1) with two major components (seed coat and cotyledon) The specific density of the seeds was measured to be about 1.2g/ml. This shows the material to be dense and the internal structure of the seed cannot have many voids.

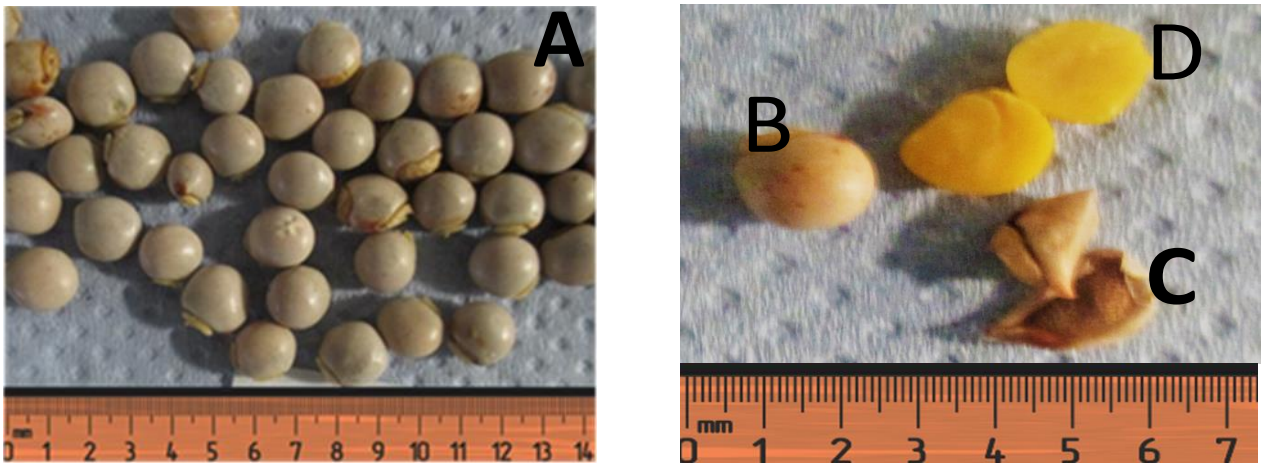



**Figure 4-1**(A) white Agatu pigeon pea seeds.
(B) not-soaked seed (C) seed coat (D) cotyledon

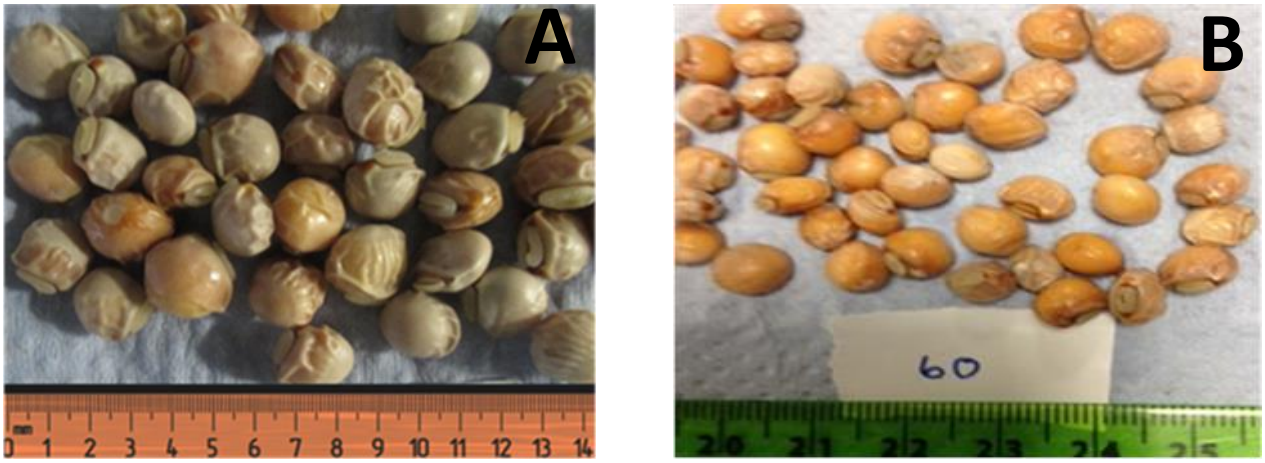



**Figure 4-2** Soaked white Agatu seed at 50 °C (A) 30 min (B) 60 min.

Within a 30 minutes of soaking pigeon pea in water at 50 °C, there is a visible change in the seed coat. It becomes wrinkled, and at this stage, it was relatively easy to remove it from the cotyledon.

**Table 4-1:** Physical properties of white Agatu pigeon pea seed.

| Physical properties | Whole seed | Dehulled seed /cotyledon | Seed coat |
|---|---|---|---|
| % weight | 100 | 88.88 | 11.12 |
| Bulk density (g/ml) | 0.811 | NA | NA |
| Specific density (g/ml) | 1.26 | NA | NA |
| Moisture content of native pigeon pea (% wwb) | 11.52 | NA | NA |
| Moisture content (% wwb) after 30 min in water at 50 °C | 16.42 | 29.33 | 57.17 |
| Moisture content (% wwb) after 60 min in water at 50 °C | 34.21 | 40.102 | 56.4 |

In Table 4-1, the relative proportion by mass masses for the cotyledon and the seed coat are given as well as the percentage (%) moisture uptake of these different seed tissues. The seed coat took up its own weight in water, while the cotyledon only increased by 29%. Hydration for seed coat removal seems to be relatively easy. However, for a legume seed to be considered safe to eat, hydration must occur throughout the seed to ensure that the thermal treatment is sufficient to reduce the anti-nutritional factors in the seed 2.2.2.

To establish whether the varieties of pigeon pea differed in their hydration properties, three different varieties were tested using the conventional soaking method. The hydration capacities of the three pigeon pea varieties (white Agatu, red Agatu, and Bayelsa) included in this research are presented in Table 4-2. Hydration capacity is a quality index and it is expressed as the percentage of water absorbed by the dry seed in relation to the dry seed weight (section 3.4.1.3). The hydration capacity was determined based on the amount of water (wet basis) absorbed by the seeds during the conventional soaking.

**Table 4-2:** Hydration capacity of white Agatu, red Agatu and Bayelsa varieties of pigeon pea seeds following conventional soaking at ambient temperature (20 °C) for 12 hours.

| Samples | White Agatu | Red Agatu | Bayelsa |
|---|---|---|---|
| 100 seed weight (g) | $11.87\pm0.08^{a}$ | $10.76\pm0.07^{b}$ | $10.26\pm0.07^{c}$ |
| Hydration capacity (%) corresponding to % increase in wet weight basis | $98\pm0.4^{a}$ | $96.8\pm0.8^{a}$ | $104.5\pm 1.2^{b}$ |

Data are mean value of the triplicate analysis ± standard deviation, values in rows denoted by the different superscript letters indicate significant differences in the values ($p<0.05$).

The results of the hydration capacity in Table 4-2 shows that all the three varieties have the capacity to absorb water at a level equivalent (100%) to their dry weight after 12 hours of soaking in water at room temperature. The highest hydration capacity was recorded for Bayelsa, followed by white and red Agatu. Meanwhile, the 100-seed weight of the samples indicated variations among the varieties with the least weight for Bayelsa (10.26 g), followed by red Agatu (10.76 g) and greatest for white Agatu (11.87 g). It has been reported that the seed weight could be either positively related to hydration capacity (Gil et al., 1996, Wang et al., 2003, Kaur et al., 2005) or negatively correlated (Wang et al., 2010) with these differences being mostly dependent on the seed structural components (seed coat and cotyledon).

It is worth noting that while Bayelsa showed the highest hydration capacity, it had the lowest dry seed weight out of the three varieties. It could be expected that the smaller the seed, the greater the ratio of seed coat to cotyledon. The seed coat of pigeon pea is of such an architecture that it is initially more permeable to water compared to the inner seed (cotyledon), as indicated in the preliminary results (Table 4-1) and reported by other authors (Singh et al., 2004, Seena and Sridhar, 2005). Possibly, the greater hydration capacity of Bayelsa indicates the presence of a large seed coat or different structural features of the seed coat and endosperm that makes

it more permeable to water. Many studies have identified that seeds with thin seed coat are more permeable to water and results in higher hydration capacity of the whole seed (Souza and Marcos-Filho, 2001, Wang et al., 2003, Tiwari and Singh, 2012, Ross et al., 2013). Whether the hydration is dominated by the ratios of the structural components or the architecture of the components is not clear. However, characteristics of the seed coat such as size, colour, porosity, chemical composition and morphology have all been implicated in water uptake by the seed (Debeaujon et al., 2000, Souza and Marcos-Filho, 2001, Ertekin and Kirdar, 2010, Ross et al., 2013). The hard to cook phenomenon may be associated with the climatic conditions and methods of drying the seeds. It may therefore not be the variety *per se* that has the major impact on hydration, but the interaction of the chemical composition of the legumes and the processing conditions to which they are subjected.

Fasoyiro et al. (2010) obtained hydration capacities ranging from 20.3-64.3% on some varieties of Nigerian pigeon pea seed after soaking at 40-70 °C for only 20-30 minutes, with highest values recorded on seeds soaked at the higher temperatures (60 and 70 °C). This suggests that seed hydration rates would be increased if seeds were soaked at higher temperatures. In contrast however, Singh et al., (2004) reported that greater weights of black gram resulted from hydration at room temperature (22 °C). Actually, the mechanism of water uptake in seeds may not be related to the seed size, but by the water permeability and absorption by the chemical components making up the seed (Williams et al., 1983) and the connectivity of the seed coat to the cotyledon cell layers. It has been reported that seed coats (testa) that are coloured may be less permeable to water because of the high phenolic content of the seed coat (Marbach and Mayer, 1974, Debeaujon et al., 2000) Some leaching of phenolic compounds into the soaking water may occur and increase the solid content of the soaking water if seeds were soaked for long periods. But red Agatu with red-coloured seed coat which would be assumed to have high phenolic

content did not show much significant difference in hydration capacity compared to white Agatu without coloured seed coat.

Generally, during conventional soaking of seeds at a temperature below the gelatinisation temperature of the starch component of the seed, it is expected that if the seed coat is permeable, water will eventually penetrate into the seed to partially soften the seed components. This allows sufficient moisture to achieve changes to the cell wall, denaturation of protein and loss of helical order in the starch at cooking temperature which allows softening of the seed during cooking (Wang et al., 2003, Güzel and Sayar, 2012). Many studies have confirmed that some of the physical characteristics of the seed, such as seed coat thickness, structural components (cell wall materials) and cotyledon components (starch and protein) affect the permeability of water into the seed (Fasidi, 1981, Mullin and Xu, 2001, Wang et al., 2003, Chaparro Acuña et al., 2012, Ross et al., 2013).

## 4.3 Chemical composition

Knowledge of the chemical composition of the seed was necessary to understand the hydration process. Also, the chemical composition of pigeon pea has been the major consideration for its human consumption, especially in developing nations (Saxena et al., 2010). Presently, consumers are more interested in consuming snacks with less starch and fat content but with more protein and dietary fibre due to the prevalent diet related diseases, which include diabetes, obesity and cardiac diseases (Brennan et al., 2013, Maskus and Arntfield, 2015). According to the methods in section 3.4.6, the levels of starch, protein and lipid were assessed for the three varieties of pigeon pea processed in three ways: whole seeds milled (NS), the whole seeds soaked, dried and milled (CS), and the whole seed soaked, dehulled, dried and milled (CSD).

T**able 4-3:**Chemical composition showing starch, protein and lipid content of pigeon pea (dwb). WA, RA and B refer to white Agatu, red Agatu, and Bayelsa. NS, CS and CSD refer to milled powder from native seed, soaked & dried seed and soaked, dehulled & dried seed. C refers to 12 h conventionally soaking applied here to differentiate from other methods of soaking applied in the next chapter. Values are means ± SD (n =3), values denoted by the different superscript letters indicate significant differences in the values ($p<0.05$) with Tukey *post hoc* test.

| Treatment | Variety | Code | Starch (%) | Protein (%) | Lipid (%) |
|---|---|---|---|---|---|
| Not soaked (NS) | White Agatu | WA-NS | $35.12 \pm 5.48^{a}$ | $20.67 \pm 1.24^{cd}$ | $0.95 \pm 0.17^{a}$ |
| | Red Agatu | RA-NS | $36.52 \pm 2.44^{ab}$ | $24.82 \pm 0.56^{d}$ | $1.36 \pm 0.59^{ab}$ |
| | Bayelsa | B-NS | $37.77 \pm 2.30^{ab}$ | $19.39 \pm 1.71^{bc}$ | $1.63 \pm 0.48^{ab}$ |
| Soaked (CS) | White Agatu | WA-CS | $36.60 \pm 2.30^{ab}$ | $18.66 \pm 0.12^{ab}$ | $1.34 \pm 0.60^{ab}$ |
| | Red Agatu | RA-CS | $38.74 \pm 0.04^{ab}$ | $17.95 \pm 2.35^{ab}$ | $1.09 \pm 0.18^{a}$ |
| | Bayelsa | B-CS | $44.41 \pm 0.00^{ab}$ | $15.63 \pm 0.19^{a}$ | $1.08 \pm 0.18^{a}$ |
| Soaked dehulled (CSD) | White Agatu | WA-CSD | $49.96 \pm 2.26^{c}$ | $17.05 \pm 0.69^{ab}$ | $2.37 \pm 0.01^{bc}$ |
| | Red Agatu | RA-CSD | $46.67 \pm 7.69^{c}$ | $20.65 \pm 1.33^{bc}$ | $2.04 \pm 0.87^{ab}$ |
| | Bayelsa | B-CSD | $49.77 \pm 1.30^{c}$ | $22.47 \pm 1.41^{cd}$ | $3.32 \pm 0.17^{c}$ |

### 4.3.1 Lipid contents affected by variety and flour preparation methods

The lipid in pigeon pea samples was assessed following the Bligh and Dyer method (1959). Interestingly, all the samples exhibited low lipid content (0.90-3.32%) compared to the higher values found in some cereals, for example 5% lipid in maize (Enyisi, 2014), 8.8% lipid in oat (Zhou et al., 1999) or some other legumes, for example 6.69% in chickpea (Chung et al., 2008). Similar to starch content, the lipid content tended to increase due to dehulling, which was indicated by the levels found in dehulled samples. But, from health and processing perspectives, due to the low lipid contents of all the varieties, pigeon pea could be beneficial in the production of the healthy extruded puffed snacks. It is known that high lipid content impact on the slippage of material in the extruder and amylose lipid complex affects expansion (Anuonye, 2012).

Finally, the lipid contents of native pigeon pea seeds from this study agreed with values given in the lipid levels for pigeon pea reported by cultivar, environment and growing location (Tiwari et al., 2008, Fasoyiro et al., 2010, Saxena et al., 2010, Okpala et al., 2013).

### 4.3.2 Starch content influenced by seed flour preparations

The assay procedure for the starch used Megazyme assay procedure and the details is given in section 3.4.6.1. For the milled pre and post soaked samples containing seed coats, not all the starch contents were statistically different whatever the variety. The values ranged from 35.15% to 44.4 % (dry weight basis) for samples that possessed seed coats. Some of these values are lower than typically reported for the pigeon pea. Ene-Obong and Carnovale (1992) reported 43.2% starch for whole seed while Yadav (1984) reported 40.54-55.14%, Saxena et al. (2010) reported 53%, though their methodologies were not reported. Ene-Obong and Carnovale (1992) reported starch content in the cotyledon alone as being greater than 50%.

The precise values for the starch will depend on the methods used for its determination.

Dehulling increased the starch content and the amounts measured ranged from 46.67 to 49.96%. This increase was a result of an increase in the cotyledon concentration where the starch is predominantly located (Saio and Monma, 1993). The increase in in starch content after dehulling were observed to be the same extent irrespective of the variety. The range of the starch content (35.15– 49.96%) measured after the different treatment was considered adequate for production of ready to eat extruded snacks (Varzakas and Tzia, 2015).

### 4.3.3 Protein content influenced by varieties and seed flour preparation methods

The protein contents given in able 4-3 show that the protein content of the native seeds determined with BCA protein assay ranged from 19.39 to 20.65%. There have been a decrease in protein level that occurred on soaking. The values are about 1.8% lower for the soaked samples when compared to the non-soaked controls and was a significant decrease ($p=0.05$) by 19.3 % for the Bayelsa variety. The leaching of soluble protein into the soak water must have contributed to the protein reduction (Rout et al., 2007, Güzel and Sayar, 2012). The protein content measured for all the pigeon peas conform to values reported for other Nigerian pigeon pea varieties, which ranged 18.34-25.21% (Yadav, 1984, Fasoyiro et al., 2010, Okpala et al., 2013). Higher values of protein were measured in red and white Agatu where the seed coat was left on the seed. The treatment of pigeon pea, especially on soaking and drying of seeds after soaking, could contribute to the variations in protein levels being reported due to loss of soluble protein and changes in protein solubility on thermal treatment. This has been reported during the drying of cowpea at high temperatures up to 80 °C (Enwere et al., 1998). The levels of protein are important as they can contribute to the nutritional quality of any snack produced from pigeon

pea. Pigeon pea could be used in the production of the extruded snacks for consumers and could be labelled as high protein if the levels of protein exceed 20% (Anton et al., 2009, Yu et al., 2013).

## 4.4 Impact of sample preparations on pasting properties of the three varieties of pigeon pea

In addition to the proximate composition, the pasting properties of pigeon peas before and after soaking were considered indicative for the quality of final products. Pasting profiles in Figure 3-3 acquired with RVA (Rapid Visco Analyser) were used to determine the pasting properties and can act as a fingerprint of starchy materials. They are used to understand the behaviour of starch granules and particles that include starch. The results are interpreted in terms of the changes in the viscosities of the native starch granules as they swell and become gelatinised and then the subsequent breakdown of the fragile swollen granule and re-ordering of the materials. The viscosities provide an insight into` the material's ability to absorb water, swell, associate and dissociate under certain temperature and shear regimes. The RVA profile parameters used in this work were defined in section 3.7.1 along with details of the method of analysis. The interpretation of pasting profiles for materials containing not only starch but also relatively high levels of protein and fibre is complex. This is because the changes of the protein, as well as interactions between the chemical components with temperature, need to be considered in addition to the starch behaviour. The lipid levels able 4-3 found for the whole pigeon pea varieties used in this PhD research were approximately 0.90-3.32% and some of the fatty acids may be associated with the starch granules. Many starches contain small amounts of lipids, typically less than 1% (Hoover, 2001) and although not high, the lipid component is known to have an impact on starch pasting profiles.

Pigeon pea pasting stirred viscosities profiles were acquired from a suspension of 12.5% solid content pigeon pea flour (with particle size

between sieve sizes 700-125 µm). The data obtained was used to indicate the suitability of pigeon pea for extrusion. The results in
Figure **4-4**-Figure 4-4-6 are presented as stirred viscosity against time with a second ordinate showing the temperature. The numerical values for all the parameters analysed from the pasting profiles have been combined into Table 4-4.

## 4.5 Pasting profile of native pigeon pea compared with wheat flour, and maize starch

It was observed from the result of preliminary studies of the pasting profiles in

Figure 4-3 that the peak viscosity of pigeon pea (627 cP), irrespective of treatment or variety, was lower than that of wheat flour (2556 cP) and maize starch (7555 cP). The suggests that limited swelling of the granule may have occurred in pigeon pea due to its difficult-to-hydrate nature (Agunbiade and Longe, 1999). However, it should be noted that the starch concentrations in wheat and maize are much greater than the 35% that occurs in the white Agatu pigeon pea (wheat flour: 77% starch, maize starch flour: 80% starch). In addition to more starch being present in the cereals, their starch granules may have a greater tendency to absorb water during gelatinisation and thus exhibit higher swelling. To understand this however the starch contents should be matched for the different samples.

(Refer to section 3.8 for features of the pasting curve and Table 4-4 for sample codes).

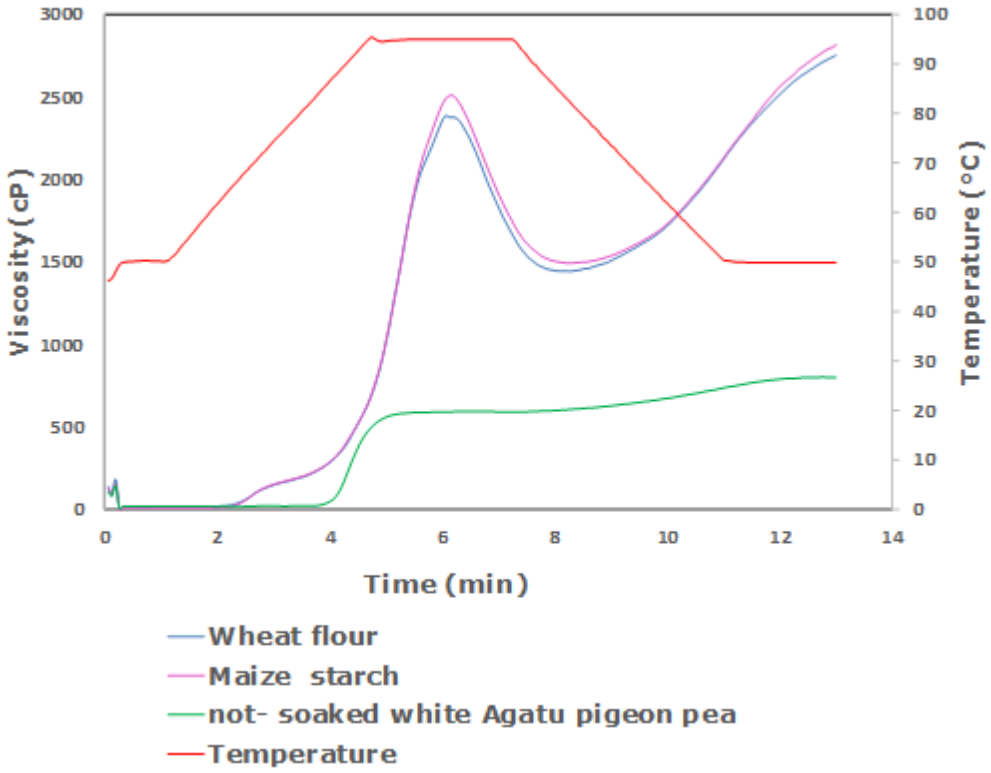



**Figure 4-3:** Comparison of the pasting profile of wheat flour, maize starch and white Agatu pigeon pea at 12.5% solid content**.**

The profile shows that the peak viscosity (which depends on water absorption and swelling capacity of the starch) of pigeon pea with broad peak was lower than wheat flour and maize starch with sharp peak viscosity. Both the absence of breakdown and setback profile in pigeon pea paste following peak viscosity is an indication of the stability of pigeon pea paste.

### 4.5.1 Not-soaked (Native seed flour) pasting properties

All the not-soaked native milled powder (NS) of the three pigeon pea varieties showed very similar pasting profiles in

Figure **4-4**. These pasting profiles are different from native wheat flour and maize starch

Figure **4-3**) at the same solid concentration (12.5 %) as there was no breakdown following the peak viscosity of the three varieties. The most important point to note is that during the pasting regimes used in the RVA (at temperatures ranging between 86-87 °C during the initial heating cycle and at times of <5 minutes) the ground powdered native pigeon pea particles (particle size between 125 to 710μm) were swelling, an indication that the starch is being cooked.

(Refer to section 3.8 for features of the pasting curve and Table 4-4 for sample codes).

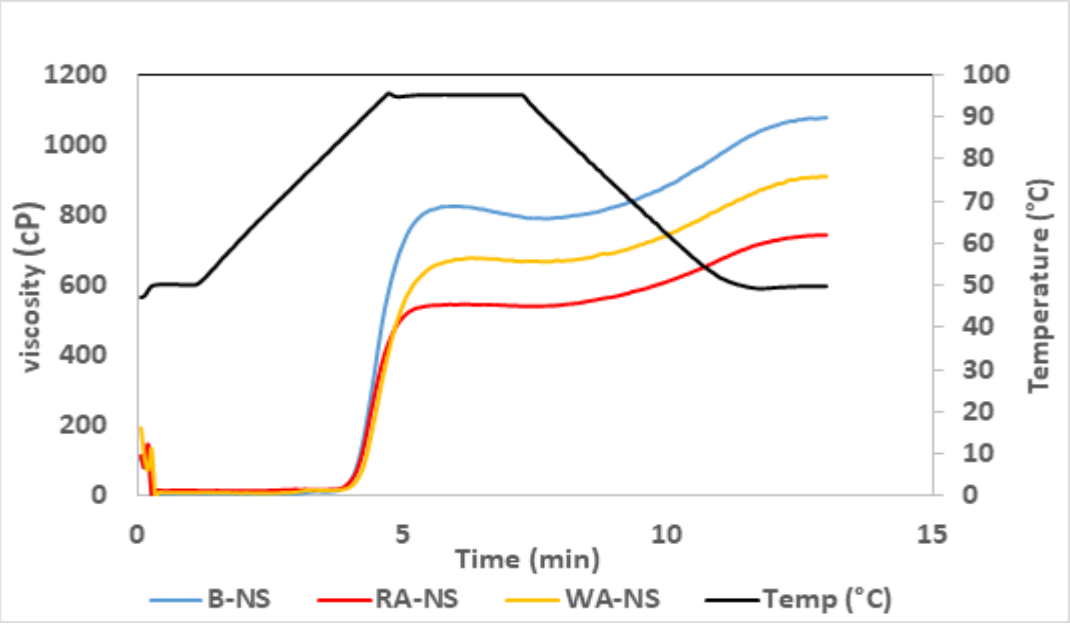



**Figure 4-4:** pasting profiles of three varieties of not-soaked (NS) pigeon pea.

Published reports on pigeon pea (flour and starch) indicated that both high (87 °C) pasting temperature (Hoover et al., 1993, Maninder et al., 2007, Lawal, 2011, Acevedo et al., 2013) and low (55 °C) pasting temperatures (Sandhu and Lim, 2008) can be required for an initial increase in viscosity of powdered suspension during pasting. Although with lower solid concentration of 11.9%, the peak viscosity of whole legume flours from pea (1371 cP), lentil (1359 cP) and chickpea (1347 cp) were found to be higher than the present studied pigeon pea (Chung et al., 2008).

Some differences between the abilities of the three varieties of non-soaked pigeon pea to hydrate and swell (peak viscosity) during pasting of the powders' suspension was observed. Although Bayelsa variety showed higher pasting viscosities (peak, breakdown and final) than the other two samples, able 4-3, it did not indicate more starch in this material. From a Nigerian consumer's perspective, Bayelsa variety is very expensive to purchase, as consumers believe it requires shorter cooking time compared to Agatu varieties. Therefore, the use of Bayelsa variety for snack manufacturing would be expensive due to the high cost of the material. If comparable performance can be obtained, farmers and consumers would prefer white Agatu due to its low cost of production and adequate availability. However, what needs to be considered as the major issue for pigeon pea is the need to soak the seed so that sufficient water is present for the starches to gelatinise and anti-nutritional factors to be denatured during cooking.

The peak viscosity that occurs in the RVA reflects the hydrodynamic volume occupied by the different seed components (starch, non-starch polysaccharides and protein) as they absorb water and swell. Generally, this peak viscosity is dominated by hydration and swelling of the native starch granules, but the non-starch components may affect the swelling of starch. From available information, the mechanism through which the non-

starch polymers influence peak viscosity of starchy materials is not clear and a different mechanisms and combinations of mechanisms may exist.

**Influence of non-starch polymers on peak viscosity**

- limiting starch hydration, reducing the moisture available to the starch
- creating a boundary around the granules so that they cannot fully swell
- Allowing interactions between the non-starch and starchy components to create structures of different hydrodynamic volumes.

For example, it was reported that removal of rice proteins (glutelin, globulin, prolamin and albumin) resulted in a decrease in the pasting peak viscosity of rice flour. In the presence of rice native protein, it was thought that the initial available water in the aqueous flour suspension could hydrate the protein and increase its solubility. This would then induce an interaction with the dispersed and viscous starch phase thereby increasing the peak viscosity (Wongdechsareku and Kongkiattikajorn, 2010). In the same way, the 20% protein within pigeon pea can be expected to have a major impact on the swelling behaviour and hence the peak viscosity.
The variations in the viscosities (peak, breakdown and final) of non-soaked pigeon pea could be due to starch interactions with non-starch components. Apart from the stability offered by the granular structural integrity, the denatured protein through aggregation and crosslinking could interact with rigid swollen granules to limit the breakdown of the paste viscosity (Singh et al., 2011). Limitations of water penetration may allow the non-gelatinised granule in the paste to achieve hydration and gelatinisation at the high temperature (95 °C) occurring during the holding period to initiate swelling, rather than granule break down (Brennan et al., 2008).

**Table 4-4**: Pasting properties of the three varieties of pigeon pea.

| Treatment | Variety | Sample code | Peak viscosity (cP) | Breakdown viscosity (cP) | Final viscosity (cP) | Pasting Temperature ($^0$C) |
|---|---|---|---|---|---|---|
| Not soaked | White Agatu | WA-NS | 627±94.4$^{a}$ | 8±4.4$^{a}$ | 854±102.8$^{ab}$ | 87±0.8$^{abc}$ |
| | Red Agatu | RA-NS | 548±50.6$^{a}$ | 8±1.7$^{a}$ | 744±61.8$^{a}$ | 87±0.1$^{abc}$ |
| | Bayelsa | B-NS | 825±37.6$^{bc}$ | 37±4.0$^{b}$ | 1076±49.5$^{c}$ | 86±0.5$^{ab}$ |
| Soaked and dried | White Agatu | WA-CS | 844±30.2$^{c}$ | 14±2.3$^{a}$ | 1138±39.7$^{c}$ | 86±0.0$^{a}$ |
| | Red Agatu | RA-CS | 596±24.3$^{a}$ | 3±0.6$^{a}$ | 803±28.6$^{a}$ | 87±0.4$^{abc}$ |
| | Bayelsa | B-CS | 688±41.6$^{bc}$ | 10±4.0$^{a}$ | 995±47.6b$^{c}$ | 88±0.0$^{d}$ |
| Soaked and dehulled | White Agatu | WA-CSD | 1618±32.0$^{e}$ | 36±6.2$^{b}$ | 2389±33.8$^{e}$ | 87±0.5$^{bc}$ |
| | Red Agatu | RA-CSD | 1473±69.9$^{d}$ | 44±11.9$^{b}$ | 1909±78.8$^{d}$ | 86±0.0$^{a}$ |
| | Bayelsa | B-CSD | 1621±10.1$^{e}$ | 52±7.2$^{b}$ | 2368±28.7$^{e}$ | 87±0.5$^{abc}$ |

Data are mean value of the triplicate analysis± standard deviation. Means with different letter in the same column differ significantly ($p<0.05$) with Tukey *post hoc* test.

### 4.5.2 Soaked and dried seed flourpasting properties

The second set of samples had all undergone a pre-soaking for 12 hours (imitating the conventional soaking of the seed before cooking) before being dried with a convection oven at 50 °C (section 3.2.3.4) and milled. Data in

Figure **4-4-5** and Table 4-4indicate that there are some differences in the pasting parameters of the soaked and dried samples compared to the non-soaked samples (NS).

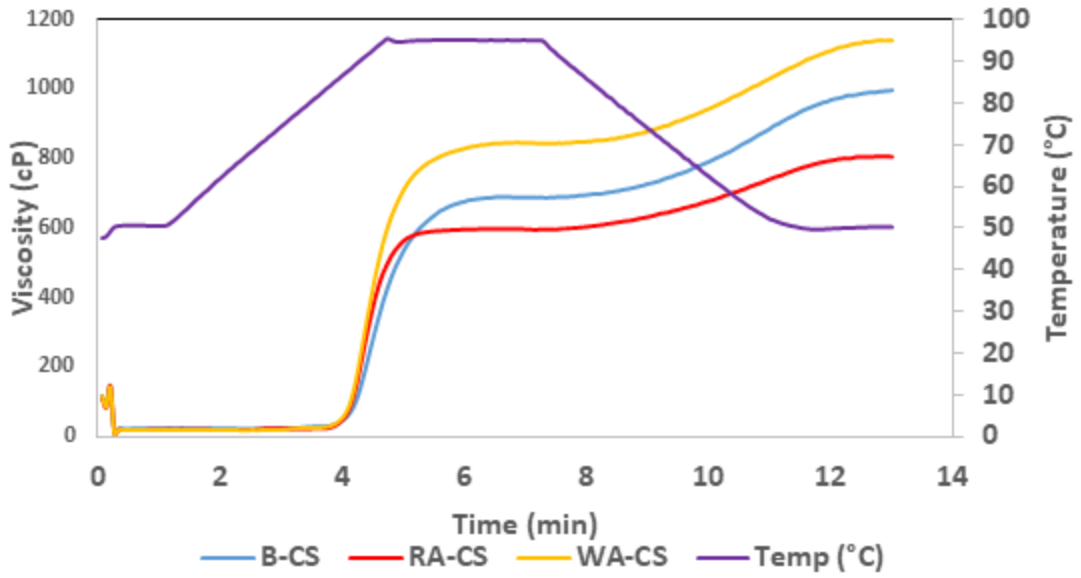



**Figure 4-4-5:**Pasting profile of the three varieties of soaked and dried (CS) pigeon pea.

The soaking at ambient temperature coupled with drying at 50 °C, may have hydrated and annealed the starch, mobilised, solubilised and decreased the protein level and finally leached salts from the seeds. These events could therefore induce changes to the materials. It is expected that the water absorbed (above 50% moisture) during the soaking of seeds would be adequate for plasticisation of the starch and possibly allow annealing. The hydration (soaking at ambient temperature) and drying condition (dried at 50 °C in convection oven) employed in this study, could

be respectively related to plasticisation and annealing respectively. The plasticisation involves the absorption of water into amorphous regions with the transition of amorphous region from the rigid glassy state to a mobile rubbery state. While the annealing of starch occurs under intermediate or excess water and temperature above glass transition (below starch gelatinisation temperature) with physical re-organisation (perfection of crystalline region) of the double helices of the starch granule and increased rigidity of amorphous region (Tester and Debon, 2000). The prior hydration of the non-starch materials may have reduced their restrictive forces for starch swelling, and hence peak viscosities may be expected to be affected. However, there is no clear impact of soaking on peak viscosities of the soaked and dried seeds.

The breakdown viscosities of the samples were similar to the native not-soaked samples. The lack of noteworthy differences between the soaked and non-soaked samples may indicate that the conventional 12 hour soaking, in combination with drying, has no permanent impact on the pigeon pea. This treatment therefore may not be an appropriate flour preparation method for the development of extruded snacks from pigeon pea.

### 4.5.3 Soaked, dehulled and dried seed flour pasting properties

The final treatment was to remove the seed coat from the soaked seeds, before they were dried and ground for pasting in the RVA (Figure 4-4-6). One factor would be that this treatment would increase the amount of starch present in the samples. The high peak viscosity of the dehulled samples (Table 4-4)compared to not-soaked and soaked samples could be attributed to the higher starch concentration of the material without seed coat. Also the increase in peak viscosity could be attributed to the increased availability of water for starch gelatinisation and swelling due absence of seed coat fibres (Tester and Sommerville, 2003).

(Refer to section 3.8 for features of the pasting curve and Table 4-4 for sample codes).

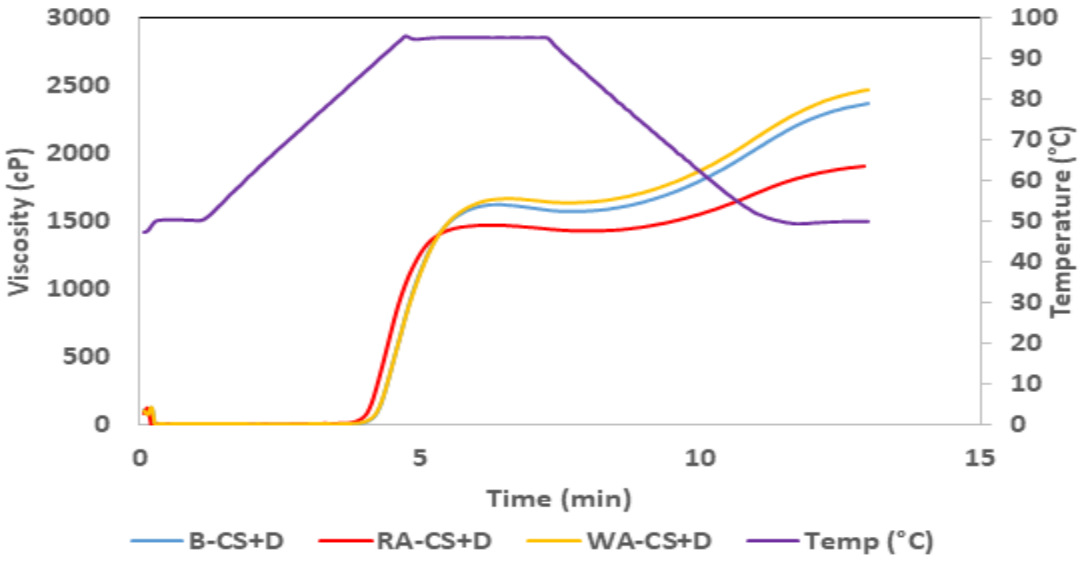



**Figure 4-4-6:** Pasting curve of the three varieties of soaked, dehulled and dried (CSD) pigeon pea.

Compared to not-soaked and soaked and dried, peak viscosity increased due to dehulling.

The recorded pasting temperature of soaked, dehulled and dried seed flours were 87.1, 86.5 and 87.6 °C, with no significant difference when compared to the not-soaked (NS) and soaked (CS) samples. If loss of ions and soluble protein occurred during the soaking and dehulling of seeds, it did not seem to have an impact on the measured pasting temperature (Figure 4-4-6) of these samples (CSD). It could be possible that changes to some of the functional properties of protein and starch, which occurred during the drying of the soaked and dehulled seeds, facilitated the significant increase in the peak viscosity of the soaked and dehulled samples. Following the drying process, the milling operations could disrupt the granules from their native matrix and thus increase starch and protein access to water required for starch gelatinisation and protein denaturation. Furthermore, it could be deduced that in the absence of cell wall materials from seed coats as in the dehulled samples, more water would be absorbed by the starch to plasticise

the amorphous region and disrupt the crystalline structure(Tester and Sommerville, 2003). All these combined effects may have resulted in the increase in the peak viscosity in addition to the relative increase in starch levels by dehulling.

Since starch concentration in the dehulled sample was higher, there was more of a tendency for the breakdown viscosity to be higher due to increase in starch concentration. With the high breakdown viscosity, the dehulled seed flour could be best used for the proposed extruded products in place of whole flour if the cost of dehulling will not inflate the cost of snack production. Both the pasting profile and pasting properties show an increase in final viscosity during the cooling of the hot paste from 1909 cP in RA-CSD to 2389 cP in WA-CSD. Probably, during the cooling of the paste, both the denatured protein and re-associating dispersed amylose reinforced the gel structural formation even though limited leaching of pigeon pea amylose has been reported (Hoover et al., 1993).

## 4.6 Chapter conclusions

As pigeon pea is reported to be difficult to hydrate and cook, some preliminary work undertaken to look at its hydration and subsequent performance identified the hydration pattern and moisture distribution within the seed major tissues (seed coat and cotyledon). The moisture content after soaking for 30 min at 50 °C indicated that native pigeon pea seed coat can take up its own weight in water while the cotyledon only increased by 29%. With this hydration pattern, it could suggest that more water is absorbed by the seed coat at the start of hydration rather than the cotyledon. This early hydration and softening of the seed coat allowed for its' easy removal.

For a legume seed to be considered safe to eat, hydration must occur throughout the seed to ensure that thermal treatment is sufficient to reduce the anti-nutritional factors and soften the seed for consumption.
The high protein content (15.63-24.82%) justifies the proposal to use the milled powder from the seed to produce healthy extruded snacks especially for developing countries where protein from animal sources is very expensive. Presumably, based on the starch content, it is possible to use the pigeon pea to produce ready to eat extruded puffed snacks. Due to the removal of the seed coat during dehulling, the starch and protein contents increased due to the increase in cotyledon fraction. Compared to cereal materials (
**Figure 4-3**), the pasting properties revealed that native pigeon pea (at the same total solids concentration) exhibited limited swelling which led to low peak viscosity and no breakdown following peak viscosity. The low peak viscosity may reflect the lower starch levels, the lower availability of water to starch during gelatinisation, the poor swelling capacity of the legume starches or even the impact of proteins on the swelling of the flour suspensions. But compared with the cereal samples, all the pigeon pea samples, irrespective of variety or treatment (soaking or dehulling), show a resistance to breakdown in viscosity when being pasted. This may confirm the thermo–mechanical stability of pigeon pea starch granules or that the starches remained robust as they never fully attained their maximum swollen volume. Therefore, it may be possible to apply higher shear and temperature during extrusion cooking without major starch degradation.

In summary, since the conventional soaking had limited impact on the pasting properties assessed for the three varieties, white Agatu, which is highly cultivated with higher productivity output, was selected and used in the subsequent sections of this study. Hydration conditions used for the work reported in this chapter did not have a major measured impact on the seeds and therefore in the following studies further actions were undertaken to enhance seed water uptake. Based on the observations of

Fasoyiro et al (2010) and Shafaei et al., (2014), which revealed that hydration capacity and hydration rate are increased at temperatures above 20 °C, the next stage of the study in chapter 5 investigated the impact of soaking at higher temperatures.

# 5 Seed softening processes prior to extrusion

## 5.1 Introduction

Considering that the 12-hour conventional soaking did not impact changes in the evaluation of the physicochemical properties of pigeon pea and due to economic consideration, the white Agatu seed, was selected for further studies. Following the selection, the seed was then subjected to several pre-treatment processes aiming to improve hydration, softening and other characteristics. To hydrate the seeds under conventional soaking, the water temperature is to be set around 20 °C, typically 12-hours is required which increases the risks of: microbial growth, loss of soluble protein from the seeds, poor production efficiencies etc. Based on literature and considering factors influencing the hard to cook defects of the seed (section 2.2.1 and 2.3.2), four different treatment methods were selected to investigate their impact on the hydration and softening of the pigeon peas seeds:

1. Higher temperature soaking (HTT) involving quiescent hydration in water bath from 30-240 min at 37 °C, 50 °C, 70 °C and 90 °C.
2. High temperature soaking (90 °C) in water for 5 min with stirring in a jacketed cooker.
3. High temperature soaking (90 °C) for 5 min in 0.5% sodium bicarbonate with stirring in a jacketed cooker
4. Enzymatic treatment of untreated whole flour and wet milled samples from treatments 2 and 3 for 1 h with 0.5% cell wall degrading enzyme (Viscozyme L, Novozyme, Denmark).

   The impacts of these treatments were evaluated through determination of functional, pasting and thermal properties, followed by assessment of crystallinity and microstructure characteristics.

## 5.2 Impact of hydrothermal treatment (HTT) at 37 °C-90 °C

To examine if soaking at higher temperatures without shear would be effective in the processing of the pigeon pea, a range of temperatures and times were studied with the following objectives: (i) To determine the optimal hydration conditions for the seeds where equilibrium (maximum) moisture content would be achieved, (ii) To reduce the 12 h prolonged conventional soaking and therefore lessen any food microbiological risks. (iii) To increase the rate of diffusion of moisture into the seed and establish the impact this may cause in terms of the changes to the seed components, (iv) To ascertain the influence of size reduction of the treated seeds by wet or dry milling.

The hydrothermal treatment (HTT) involved hydration of the seeds at 37 °C, 50 °C, 70 °C and 90 °C, in a quiescent condition, for up to four hours. The weight gained, on a dry matter basis, was measured at intervals of 30 min (section 3.2.2.1 for detailed methodology). To establish if the hydration process at 37, 50, 70, 90 °C and wet milling caused irreversible changes, the hydrated samples were dried and milled and results were compared. The textural quality, pasting, thermal and microstructural characteristics of the wet and dry milled samples were analysed, and their results are discussed in the appropriate sections.

### 5.2.1 Water uptake during HTT at 37-90 °C

On adding 11% moisture content (dwb) pigeon pea seeds to water, preheated to a range of temperatures (37-90 °C), the seeds absorbed water. During these hydrothermal pre-treatments, the trends in water uptake shown in Figure 5-1 was expressed as % (percentage) moisture content on a dry weight basis (dwb). The method for the calculation of water uptake in this present hydration treatment(HTT) was different from the previous hydration capacity discussed in chapter 4 in which the water absorbed by the seed (hydration capacity) was related as % increase in wet weight basis (wwb).

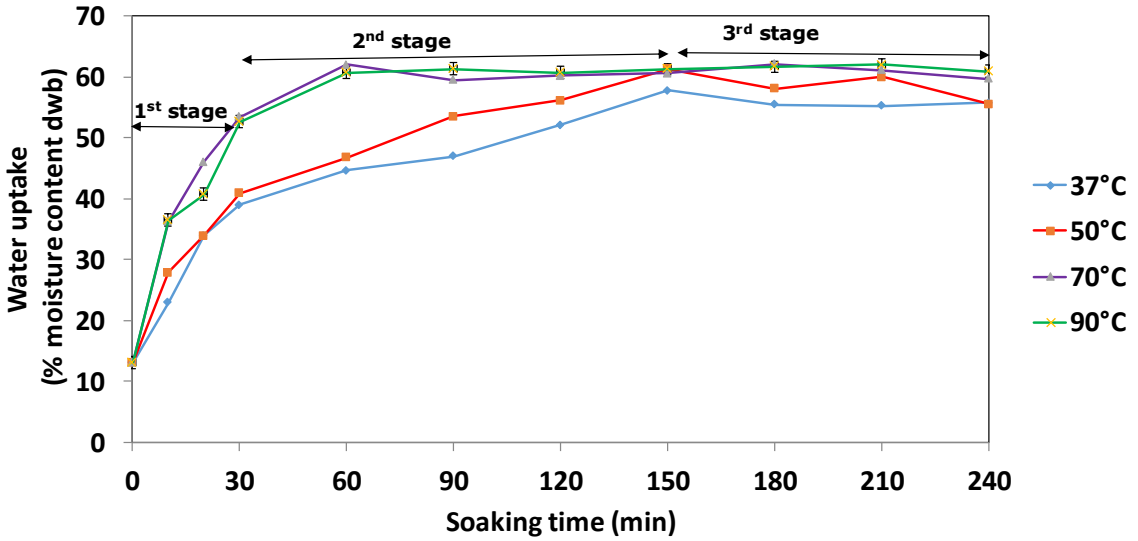



**Figure 5-1:**Water uptake during hydration (HTT) of seed at 37-90 °C for 240 min.

First stage: involvement of seed coat in the rapid water uptake, 2nd stage: temperature dependant swelling of seed, 3rd stage: lag stage with minimal changes in seed water uptake.

Based on the observation made on the water uptake curve in Figure 5-1 and physical changes on the seed, there appeared to be three stages during the water uptake. First, there was a rapid water uptake up in which the seed coat seems to be the primary factor followed by a second stage where is a moderate increase, that looks as if the temperature makes a marked difference to the rate and then a final period where the increase in water uptake are small. In the first stage, a marked increase in water uptake within the first 30 minutes of soaking was observed possibly by diffusion action. The initial moisture content of the dry native seeds increased from 11% to a maximum of 52.7% at 90 °C and this caused the seed coat to soften and collapse. This effect occurred regardless of soaking temperatures. This initial rapid water uptake without a lag (time before a change is seen in water uptake curve) is an indication of the permeability (presence of porous structure) of the seed coat, a factor suggesting that pigeon pea is a well hydrating seed (Ross et al., 2013). The permeability of the seed coat has been identified as the major factor controlling the uptake

of water into the seed (Souza and Marcos-Filho, 2001, Paiva et al., 2006, Ross et al., 2013).

A consequence of the rapid uptake of water by the seed coat (through diffusion actions) is that it becomes softer and wrinkled in the first 30 min of soaking. Rapid swelling of the seed in the second stage, which intensified as water uptake increased (40-60%), follows this. Faster water uptake occurred because of the increase in temperature from 37 to 90 °C and time from 30 to 60 min. Changes in diffusion kinetics were expected as temperature and time influences the increase the water uptake, and plasticisation of the cotyledon cellular components (starch, protein, cell wall materials). In addition, because of changes to the physical chemistry of the matrix materials, it seems that once the seed coat is hydrated, the seed's moisture content continued to increase though is much affected by the temperature.

More water may be entering the cotyledon through the intercellular spaces by capillary action Previous work shows that seed weight after 12 hours soaking at ambient temperature (~20 °C) increased by 98 %(Table 4-2). The plateau values for hydration of the white Agatu seeds appeared at approximately 60% moisture content (dwb), considerably less than achieved for the longer period (12 hour) at lower temperature (~20 °C). Irrespective of temperature and time, the seed reached the maximum hydration capacity where it has absorbed maximum water (equilibrium moisture content) in the range of 57.8 -62.34%. From water uptake graph in Figure 5-1, it shows that seeds soaked at lower temperatures (37 °C and 50 °C) obtained an equilibrium moisture content of 57.8% and 61.3% respectively at 150 min. However, seeds soaked at 70 °C and 90 °C obtained 62.34% and 60.71% of equilibrium moisture content respectively at a shorter time of only 60 min.

The third stage in the water uptake curve was a lag stage with neither increase in the hydrated seed moisture content. Although minimal physical

changes occurred in the seed but more leaching of solute occurred (Figure 5-2) due to the disruption of cell membrane integrity (Powell, 1986).
Although the equilibrium moisture content was approximately the same with minimal impact of temperature, a higher soaking temperature of 70 °C and 90 °C, decreased the equilibrium time by over twofold compared to the time required for low soaking temperatures of 37 °C and 50 °C. At higher temperatures (70 and 90 °C), it was thought that the possible gelatinisation of starch could cause more water to be absorbed by the seeds, but there was no evidence that the higher temperatures had an impact on the moisture levels. It was also possible that some starch gelatinisation may cause a reduction in water absorbance rates as the cooked starch could block the interstitial spaces between the cells.

The short times (60 and 150 min at different temperatures) required to reach the equilibrium moisture content during this HTT contrasts with the 12 hours conventional soaking at room temperature, which traditionally, consumers of pigeon pea considered necessary for the maximum water absorption and softening of the seed prior to cooking. Although soaking at 20 °C for the longer time (12 hours) could make more solute available from the leaching of some soluble anti-nutritional factors, but the problems associated with increasing the microbial load while hydrating pigeon peas makes the faster option of using the higher temperature more acceptable.

Based on the optimal conditions surrounding equilibrium moisture content of the present hydration method, an ambient soaking condition at 37 °C; 150 min was selected, which is appropriate for the seed to reach the equilibrium moisture content of 60% (dwb) compared to the conventional 12 hours soaking.

### 5.2.2 Electrical Conductivity (EC)

As moisture goes into the pigeon peas and plasticises their cell walls and other components, the constituent particles may be solubilised and leach into any free water. One of the factors established in chapter 4 was that proteins may be lost in the soaking procedure used to hydrate the pigeon peas. If soluble proteins can be lost, then salts and other nutrient materials may also be leached from the seeds. To investigate this, the conductivity of the fluid in which the seeds were soaked was measured. The method used to follow this is given in Section 3.4.1.5. The electrical conductivity (EC) was determined to assess the leachable solutes during the HTT, which is an indication of loss of cell membrane integrity. Figure 5-2 shows that at higher soaking temperatures (90 °C) and time, more solutes were leached out due to increase in softening of the seed and the loss of cell integrity (membrane). This also corresponds to the high reduction in the force required to deform the seed.

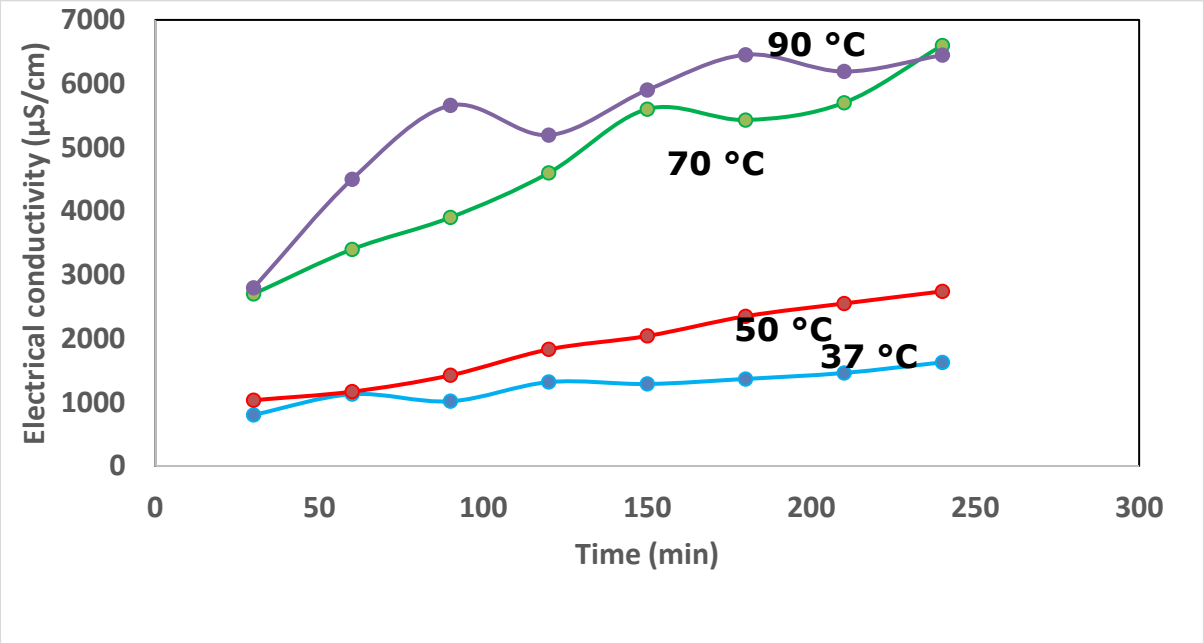



**Figure 5-2:** Electrical conductivity (EC) following hydration (HTT) of the seed 37, 50, 70 and 90 °C.

The increase in the electrical conductivity (EC) ranged from 1630 µS/cm at the lowest soaking temperature of 37 °C to 6450 µS/cm at the highest

soaking temperature of 90 °C. This implies that soaking at higher temperatures influenced the mass transfer of water into the cell and solvation of cellular material that can diffuse out due to the rupture of seed cell membrane (Duke et al., 1983). The damage to the membrane results in leaking of salts, soluble protein and formation of gel layers from the seed mucilage, which is said to reduce diffusion of water although that was not observed in the water uptake measures. Most of the leaking solutes have been reported to come from the seed coat and tissues associated with seed coat such as the surface of the cotyledon (Duke et al., 1983, Fessel et al., 2006, Nasar-Abbas et al., 2008). The lower EC at 37 °C may be linked to the lower penetration of water into the structural and cellular components, which affects the solubilisation of soluble components. Hence, both the seed coat and cotyledon were not hydrated enough to cause membrane damage and more leaching of solutes. It has been reported that the degree of permeability of the structural and cellular components determines the extent of the EC of the soaking water (Matthews and Powell, 2006).

The trend in the EC curve after hydrothermal treatment could be related to the seed water uptake behaviour in Figure 5-1. The initial rapid increase in EC corresponds to the initial rapid increase in water uptake at corresponding temperature due to osmotic and matrix potential (Duke et al., 1983, Plhak et al., 1989, Matthews and Powell, 2006). The steep fluctuation in the EC curve of 70 °C and 90 °C after the initial increase corresponds to the period of the equilibration of water uptake (equilibrium moisture content). This steep fluctuation in EC curve indicates that at equilibrium water uptake, where maximum water is absorbed by the seed, the leaching of the solutes into the soaking water will be equilibrating to maximum EC. Further than this point as similar to the lag stage (third stage) in hydration curve in Figure 5-1, the leached solute may increase the viscosity of the soaking water and delay diffusion of water into the cells with the consequence of restricting leaching of solute.

Given that leaching of solutes and electrolytes from the hydrated biopolymers (protein, starch) and certain minerals from the seed are detrimental to the nutritional values and leaching is increased with soaking temperature and water uptake, it could be argued that it is more appropriate to soften the seeds at higher temperature (90 °C). Hence benefiting from shorter soaking time. However, the longer period of soaking may increase the leaching of more soluble anti-nutritional compounds such as phytates, tannins and the raffinose family of oligosaccharides.

### 5.2.3 Textural quality of seeds after HTT at 37-90 °C

One feature of hydration is that the rigidity of the pigeon pea seed changes. To investigate the change in hardness the deformation force was measured. The deformation force is the force that causes cell separation as the probe penetrates (puncture) 60% of the seed's cross-sectional diameter according to the method described in Section 3.4.4. The deformation force also relates to the resistance of the seeds to structural degradation and is relevant, as the samples will require milling as part of the processing procedures. Figure 5-3 to Figure 5-4shows the impact of hydration on deformation force. The un-soaked samples (NPP) had peak deformation forces of 12 ± 3.66 N thereby showing the highest deformation forces compared to the other samples. The deformation force decreased as the soaking time increased from 2 hours to 4 hours and temperature increased from 37 °C to 90 °C.

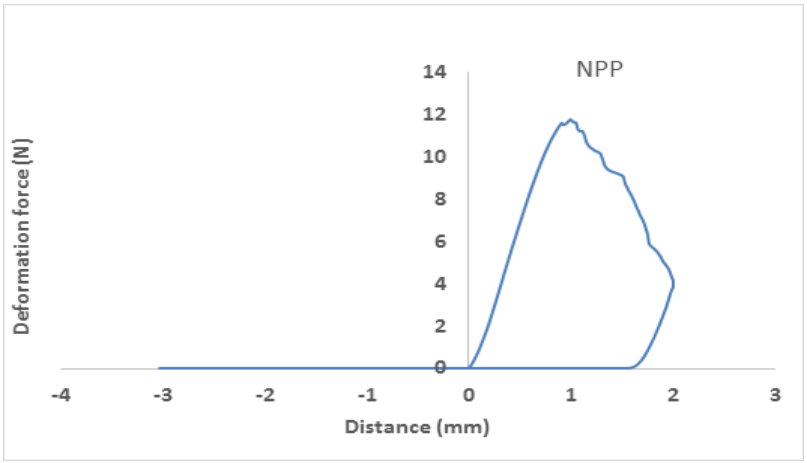



**Figure 5-3:** Deformation force curve of native un-soaked seed (NPP).

High deformation force was required to puncture NPP seed due to the rigidity of cellular structure following the non-soaking of the seed.

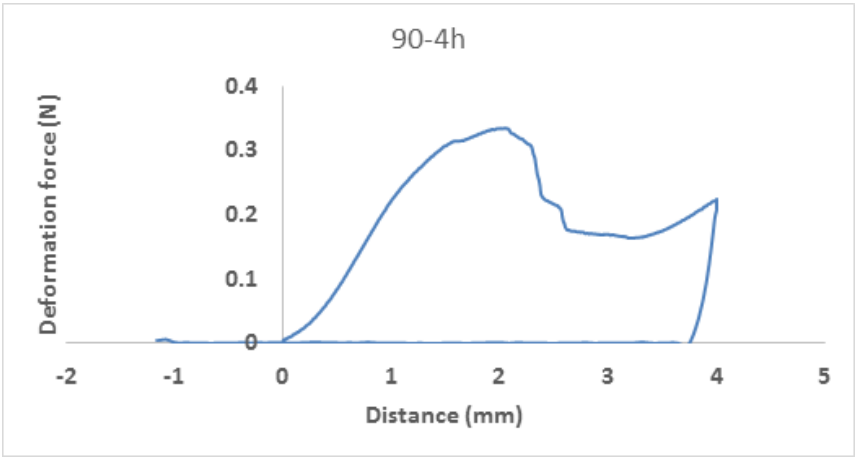



**Figure 5-4:** Deformation force curve after hydration of the seed at 90 °C for 4 hours.

Considering the high hydration temperature and long-time (4 h), starch gelatinisation did occur with subsequent softening of the seed and reduction in deformation force.

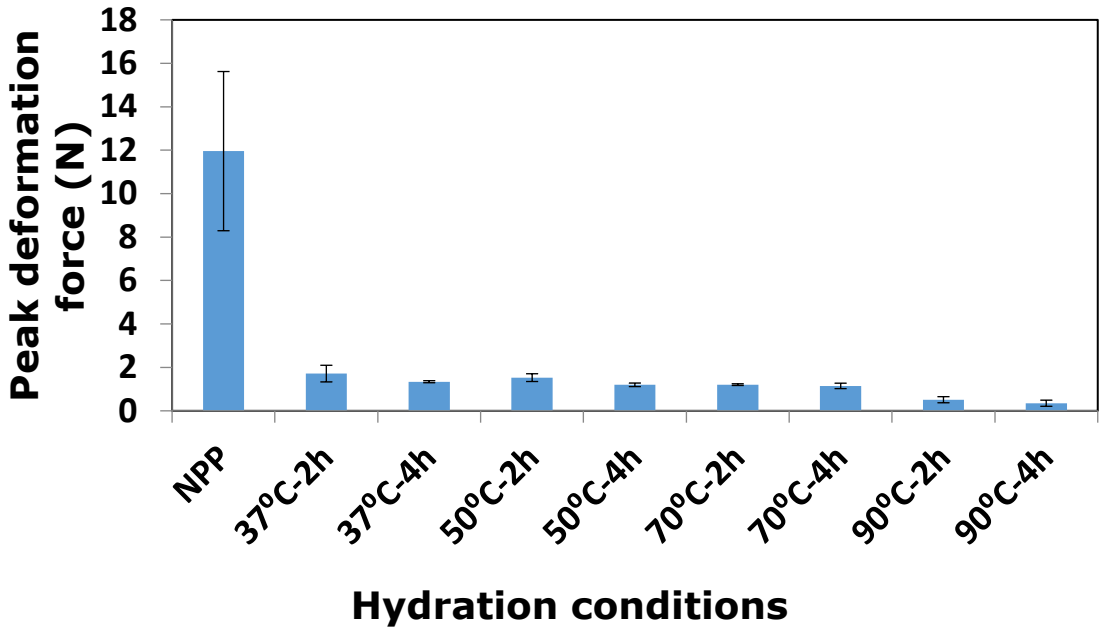



**Figure 5-5**: Peak deformation force of native and soaked pigeon pea seed after soaking in water at 37, 50, 70 and 90 °C for different times (2 and 4 hours).

Error bar represent ± standard deviation of ten replicates. The soaking temperature is displayed in °C while the initial soaking time is in hours (h).

Notwithstanding that from the water uptake curve in Figure 5-1, the equilibrium moisture content acquired by all the samples were relatively similar, the trend in the peak deformation force required to deform the seed was different. Highest reduction of deformation force (97%) was reached after soaking at 90 °C for 4 hours compared to NPP. Although the moisture content was about the same in the 70°C and 90 °C samples but there was a greater reduction of seed deformation peak force when soaked at 90 °C. At this highest temperature (90 °C), the consequence of thermal degradation (softening) of seed components (seed coat and cotyledon cellular components) is the softening and separation of cotyledon cells. The softening effect may be impacted by the gelatinisation of starch, denaturation of protein and degradation of cell wall materials. At 90 °C and equilibrium moisture content of the soaked seeds, the starch could be expected to lose order. The gelatinised starch does not seem to encourage uptake of water, presumably as it lacks space within the cellular matrix to

swell. The loss of starch order and possibly that of proteins and cell wall materials could be the cause of the seed softening.

The results further revealed that the reduction of hardness was lower when seeds were soaked at a temperature below the starch gelatinisation temperature (37 to 70 °C) compared with when they were soaked at 90 °C. At the lower temperatures, the cell walls and cotyledon cellular components could still be un-degraded with structural integrity. An insignificant difference ($P<0.05$) in the deformation force occurred among samples soaked at low temperatures (37 °C and 50 °C) though these samples had similar equilibrium moisture contents. This may indicate that in this moisture (57.8-62.34%) and temperature range (37 to 70 °C), the material gets softer due to dilution and plasticisation by water, without any major structural changes. Similar deformation forces are therefore required for the penetration of probe, but when compared with NPP a significant reduction in deformation force occurred.

At this stage of textural assessment, the effect of these hydration methods (HTT) on the seed structure and components is not fully understood, but two things could be considered. (1) The outside of the seed may be hydrated while the central core may not be, and the deformation forces may reflect this. (2) There may be enough hydration at the outside to allow changes in the starch and protein, but nothing was changing in the centre. Therefore, the softening of the seed that occurred at 90 °C soaking temperature, could be due to thermal degradation of cotyledon cellular components, and below this temperature the cell wall and cotyledon would still be rigid requiring more deformation force would be required to puncture the seed hence higher value would be recorded.
To understand the real effects of HTT on the degradation of cotyledon structural components, especially on the starch conversion, further experiments are necessary. Starch structural analysis can be conducted with these analytical techniques such as rapid visco analysis (RVA) pasting

properties, differential scanning calorimetry (DSC), wide angle X-ray diffractometry and microscopy.

### 5.2.4 DSC thermal properties after HTT at 37-90 °C

One way of establishing if the starch crystallinity and molecular order had been altered by the hydrothermal treatment (HTT) was to measure the gelatinisation temperature and enthalpies of the processed samples and compare these with the non-processed control. The differential scanning calorimetry (DSC) method was used for the measurement of the gelatinisation properties and details of the method is given in Chapter 3 section 3.7.3. For this evaluation only the wet and dried milled samples obtained after 4 hours of hydrothermal treatment were investigated. The results are shown in Table 5-1 and
**Figure 5-6** to Figure 5-7. The 90 °C HTT for 4 hours samples showed no detectable gelatinisation enthalpy, indicating that all the crystalline and molecular order were dissociated during the high temperature hydration. However, there was no significant difference in enthalpies of HTT at 37 °C to 70°C (5.51-7.27 J/g) compared to 6.48 J/g of native pigeon pea (NPP). These enthalpies are higher than similar whole flours from some legumes such as pea 4.5-4.8 J/g, lentil 3.0-3.2 J/g and chickpea (4.3-5.1) (Chung et al., 2008). Suggesting that HTT at 37 °C to 70°C and longer soaking time of 4 hours had no impact in reducing the amount of energy required to gelatinise the granule. This in an indication that both the melting of the starch crystallite and the unfolding of its double helical structure do not occur at temperatures below starch gelatinisation, no matter how long a starchy material is being exposed to such conditions.

**Table 5-1:** DSC thermal properties of wet and dry milled HTT at 37 °C to 90 °C.

| Sample abbreviation | Milling technique after soaking | Soaking temp(°C) | Soaking time (hours) | Onset gelatinisation temp(°C) | Peak gelatinisation temp (°C) | Endset gelatinisation temp(°C) | Gelatinisation enthalpy(J/g) |
|---|---|---|---|---|---|---|---|
| NPP | Dry | NA | 4 | 76.26$^{a}$ | 84.15$^{a}$ | 99.53$^{a}$ | 6.48$^{a}$ |
| WM37°C-4h | Wet | 37 | 4 | 79.74$^{c}$ | 85.35$^{a}$ | 97.76$^{a}$ | 5.61$^{a}$ |
| DM37°C-4h | Dry | 37 | 4 | 79.34$^{bc}$ | 85.41$^{a}$ | 98.65$^{a}$ | 5.65$^{a}$ |
| WM50°C-4h | Wet | 50 | 4 | 78.94$^{bc}$ | 84.87$^{a}$ | 96.81$^{a}$ | 7.29$^{a}$ |
| DM50°C-4h | Dry | 50 | 4 | 79.26$^{bc}$ | 85.15$^{a}$ | 98.41$^{a}$ | 5.75$^{a}$ |
| WM70°C-4h | Wet | 70 | 4 | 78.25$^{b}$ | 84.72$^{a}$ | 100$^{a}$ | 6.57$^{a}$ |
| DM70°C-4h | Dry | 70 | 4 | 79.06$^{bc}$ | 84.55$^{a}$ | 98.26$^{a}$ | 6.10$^{a}$ |
| WM90°C-4h | Wet | 90 | 4 | ND | ND | ND | ND |
| DM90°C-4h | Dry | 90 | 4 | ND | ND | ND | ND |

Soaking temperature (°C) and time in hour (h) while WM: wet milled and DM: dry milled. Data are mean value of the duplicate analysis± standard deviation. Means with different letter in the same column differ significantly ($p<0.05$) with Tukey *post hoc* test.

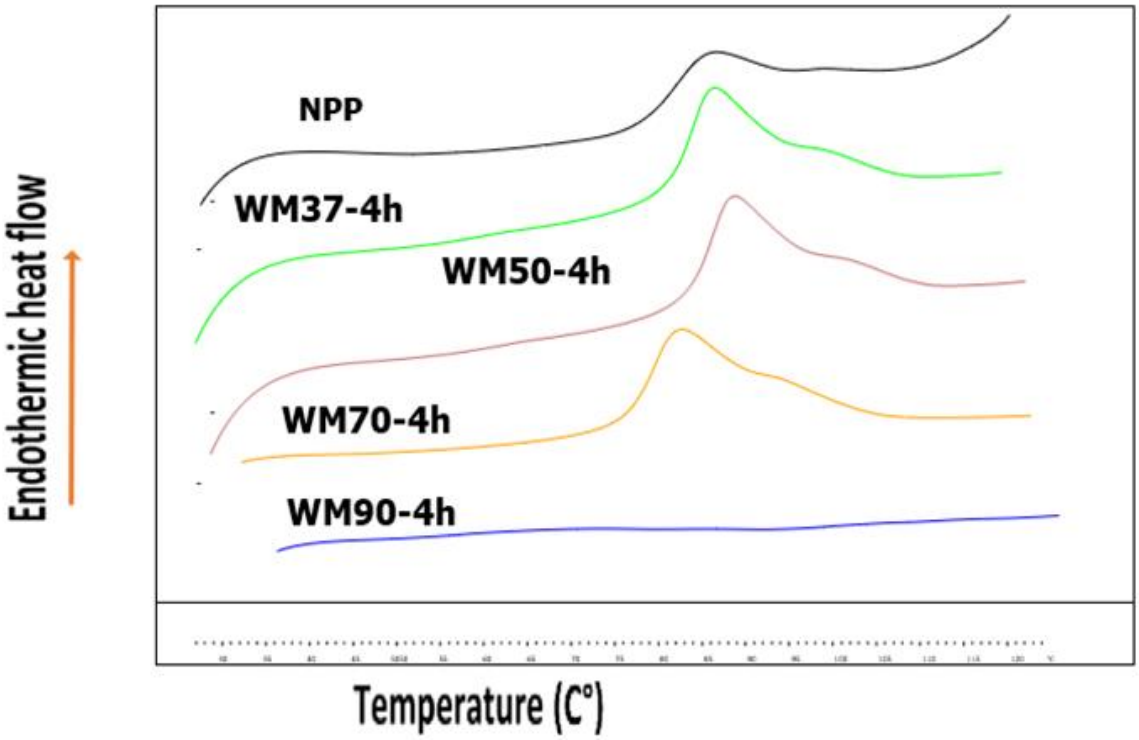



**Figure 5-6:** DSC profile after hydrothermal treatment at 37 °C to 90 °C and wet milling**.**

Due to loss of starch crystallinity and molecular order, no endothermic peak was detected in sample WM90°C-4h. Soaking temperature (°C) and time in hour (h) while WM: wet milled.

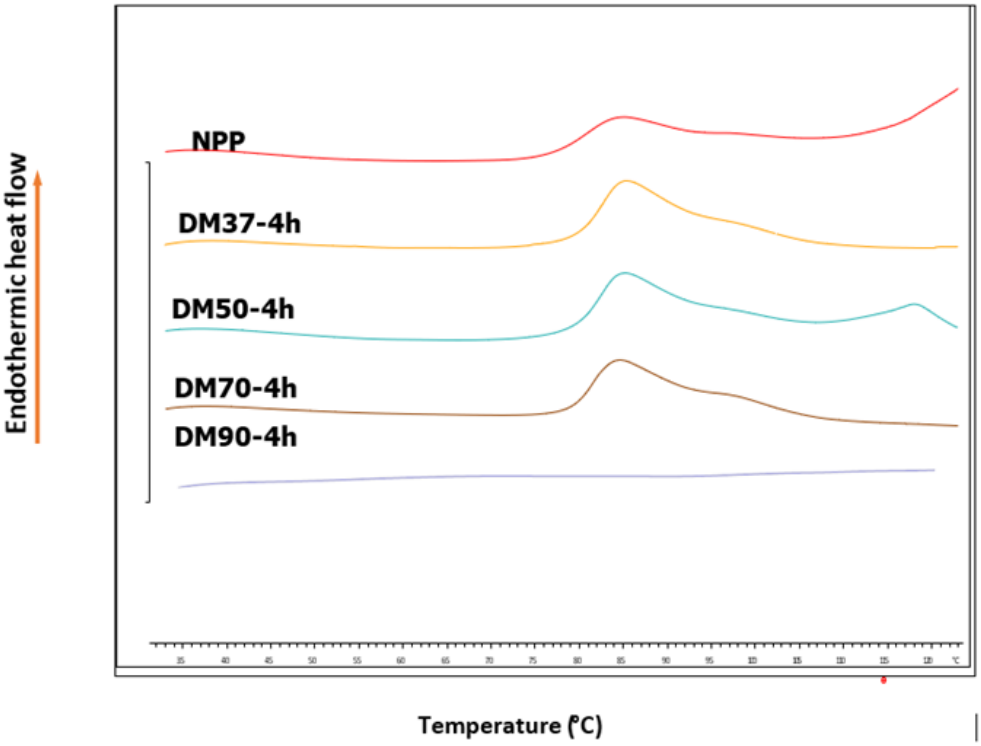



**Figure 5-7:** DSC profile after hydrothermal treatment at 37 °C to 90 °C and dry milling**.**

Similar to WM90°C-4h, the absence of endotherm in DM90°C-4h confirms that hydration at higher temperature and time resulted in starch gelatinisation (loss of crystallinity and molecular order). Soaking temperature (°C) and time in hour (h) while DM: dry milled.

Although the conventional soaking increases the hydration and moisture content, the samples only showed starch cooking at temperatures, times and moistures applicable in the 90 °C HTT for 4 hours. So long cooking time for the cooking of pigeon pea seed is all about waiting until the hydration of starch occurs and then achieving sufficient temperature so that the starch will cook. If pre-hydrated, then the starch will cook quickly at the gelatinisation/cooking temperature. Based on the difference in the enthalpy required to cook the starch, it then means that mass diffusion of starch is slow during the conventional soaking, whereas heat transfer is much quicker during cooking at higher temperature. Therefore, processing of pigeon pea will be more efficient at the gelatinisation temperature if hydration has first occurred.

DSC also allows an insight into the temperatures at which the change occurs in the starch granule. Compared to NPP (Table 5.1), there was a significant difference ($P<0.05$) in the onset gelatinisation temperature following HTT at 37 °C to 70 °C. The HTT at 37 °C to 70 °C resulted in higher onset temperatures. In contrast to the differences between the onset temperature of native un-soaked sample (NPP) and HTT samples of 37 °C to 70 °C, the endset (conclusion) temperature of NPP did not shift from HTT at 37 °C to 70 °C (Figures 5.6 and 5.7). The onset gelatinisation temperature of all the HTT samples (wet and dry milled) ranged from 78.24-79.73 °C while NPP was 76.26 °C. The higher values of onset gelatinisation temperature after HTT at 37 °C to 70 °C is an indication that more stable crystallite were formed due to the annealing of starch during the HTT and oven drying, which occurred at temperatures below pigeon pea starch's gelatinisation temperature. Similar effects have been reported during annealing of some starches (Tester and Debon, 2000, Adebowale et al., 2009). These thermal processes (hydration and drying) at below 90 °C could perfect pigeon pea starch crystalline structure without destroying it compared to HTT at 90 °C for 4 hours which caused complete loss of the starch macromolecular structure. Hence the consequence of starch gelatinisation (loss of crystalline and molecular order) following HTT at 90°C for 4 hours is the softening of the seed and high reduction in peak deformation force. This contrasts with hydration at low temperature (37-70 °C) which though caused a minimal reduction on peak deformation force but without change in the enthalpy of starch gelatinisation due to the hydration temperature was below starch gelatinisation temperature.

### 5.2.5 Effect of 37-90 °C HTT on pasting properties

#### 5.2.5.1 Introduction

Some proposed scenarios that may have affected the starch swelling behaviour during the HTT and the subsequent pasting properties are hereby discussed. The first of the three scenarios was that the starch granules may

remain native after the HTT treatment at higher temperature if the water that goes into the cell did not hydrate the starch. In the second scenario, the granule having been hydrated could not gelatinise at appropriate temperature due to limited shear. In the third, the starch will be cooked, but remains intact within the cell. In the fourth scenario, the outside of the seed's cotyledon will be cooked due to rapid starch gelatinisation, but in the internal regions, the starch within the cells remained native.

Generally, the most important thing about starch, is the loss of crystallinity. when it is cooked. Although the DSC gives some indication of the starch thermal behaviour, and can answer which of the scenarios is likely to occur, but cellular integrity and swelling of the materials when encased in the cell wall is hard to predict. Microscopy helps to form a picture of the structures, but how they may interact in water is not easily discernible.

To further investigate the cooking behaviours of the 37-90 °C HTT samples, they were ground and heated in excess water and the resultant viscosity followed. The viscosity of the paste, which developed during cooking, shearing and cooling of wet milled and dry milled flour suspension was assessed with a Rapid Visco Analyser (Newport Scientific, Australia). The pasting curves are shown from Figure 5-8 to Figure 5-10, while the pasting properties are presented in Table 5-2. The features of the pasting curve are described in section 3.7.1. The impact of HTT on the wet and dry milled samples were compared in addition to the untreated native sample. The choice of the HTT samples selected for this evaluation was based on the samples at equilibrium moisture content time and beyond the equilibrium moisture content time (2 h and 4 h). The result of RVA will be able to reveal

if further soaking of the seeds at these soaking conditions influences the starch granule integrity and particulate suspension cooking properties.

#### 5.2.5.2 Effect of high HTT (90 °C) on pasting properties

Figure 5-8 shows the WM 90 °C samples on a magnified scale, but no major swelling event is obvious. This could have been expected as the DSC had indicated that all the starch was gelatinised and therefore the completely amorphous starch could not have been expected to hydrate and swell at 50 °C (lowest pasting temperature) when exposed to excess water.

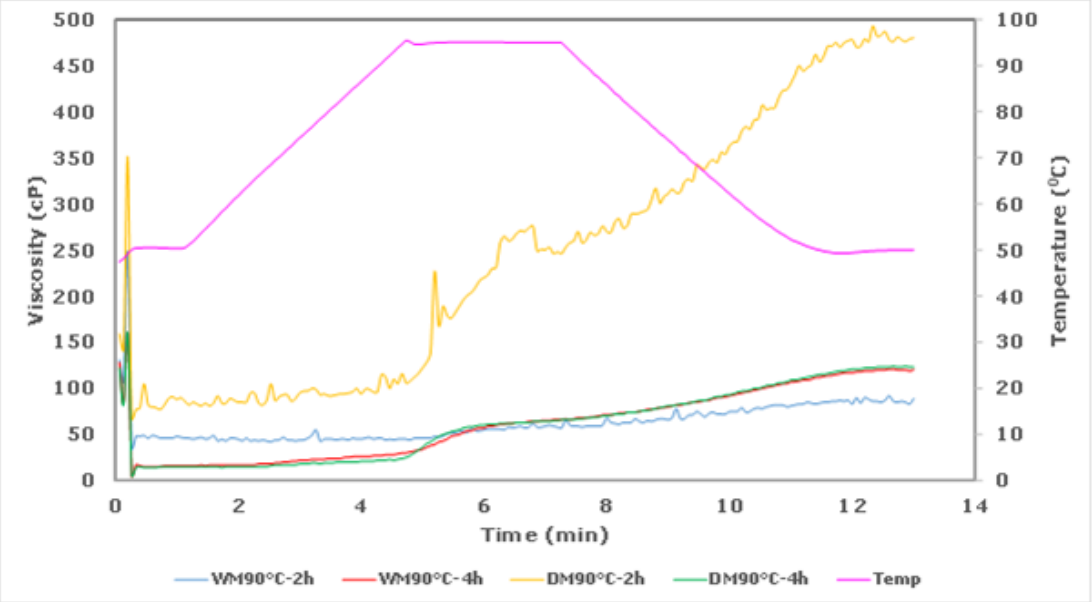



**Figure 5-8:** Effect of high temperature (90 °C) HTT on pasting profile.

The limited cold water viscosity at 90 °C and hot paste peak viscosity indicates the total disruption of starch granular integrity as amorphous starch cannot swell under these pasting conditions. The figure legend indicates soaking temperature (°C) and time in hour (h) while WM: wet milled and DM: dry milled.

There are several reasons why a pre-gelatinised starch may not swell when exposed to excess water at 50 °C. It is possible that the starch after HTT at 90 °C has reformed some structure (retrograded), and this needs to be melted before cold water swelling can occur. However, the DSC showed no enthalpies of WM 90 °C samples and if amylopectin retrogradation had

occurred it would have been detected by this method. Amylose retrogradation or the formation of amylose-lipid complexes are more difficult to see in the DSC thermograms and these may have been why the WM 90 °C wet milled particles could not swell at lower temperature (50 °C).The encapsulation of the starch within the cell matrix may also prevent starch swelling during pasting (Hasjim et al., 2013). Although in WM-90 °C, the starch has been cooked but the interaction of starch with the protein may be relevant. The protein-starch mixture, in which the starch has lost its crystallinity structure, may be difficult to hydrate.

**Table 5-2:Pasting properties of pigeon pea following 37-90 °C HTT and milling.**

| Sample | Peak viscosity (cP) | Final viscosity (cP) | Setback (cP) |
|---|---|---|---|
| NPP | 663±44.80$^{ab}$ | 843±45.53$^{abc}$ | 204.33±8.74$^{bc}$ |
| WM37°C-2h | 3208±16.45$^{d}$ | 3593±16.55$^{e}$ | 852±56.03$^{f}$ |
| WM37°C-4h | 2098±14.68$^{c}$ | 2454±15.62$^{d}$ | 554±47.79$^{de}$ |
| DM37°C-2h | 715±7.07$^{ab}$ | 964±19.09$^{abc}$ | 260±11.31$^{bc}$ |
| DM37°C -4h | 898±57.42$^{b}$ | 1148±54.01$^{c}$ | 264±2.52$^{bc}$ |
| WM50°C-2h | 1700±50.8$^{bd}$ | 2281±47.04$^{d}$ | 652±31.07$^{e}$ |
| WM50°C-4h | 2257±26.51$^{c}$ | 2820±28.59$^{de}$ | 692±60.74$^{ef}$ |
| DM50°C -2h | 710±17.21$^{ab}$ | 1010±24.43$^{bc}$ | 332±11.55$^{bc}$ |
| DM50°C -4h | 804±31.90$^{ab}$ | 1037±39.93$^{c}$ | 247±9.50$^{bc}$ |
| WM70°C-2h | 3377±90.82$^{d}$ | 4590±11.61$^{f}$ | 1320±22.13$^{g}$ |
| WM70°C-4h | 4821±36.29$^{e}$ | 6666±39.69$^{g}$ | 1945±38.53$^{h}$ |
| DM70°C -2h | 611±27.74$^{ab}$ | 957±41.33$^{abc}$ | 415±13.61$^{cd}$ |
| DM70°C -4h | 500±6.35$^{ab}$ | 959±16.82$^{abc}$ | 548±14.29$^{de}$ |
| WM90°C -2h | 264±19.34$^{ab}$ | 492±24.90$^{abc}$ | 266±9.01$^{bc}$ |
| WM90°C -4h | 61±1.09$^{a}$ | 93±4.54$^{a}$ | 24±8.86$^{a}$ |
| DM90°C -2h | 62±1.52$^{a}$ | 119±3.51$^{ab}$ | 60±1.15$^{a}$ |
| DM90°C -4h | 66±1.52$^{a}$ | 126±5.50$^{ab}$ | 66±4.93$^{a}$ |

Soaking temperature (°C) and time in hour (h), wet (WM) or dry milled: DM, NPP: native non-treated pigeon pea. Data are mean values of the triplicate analysis ± standard deviation. Means with different letters in the same column differ significantly ($p<0.05$).

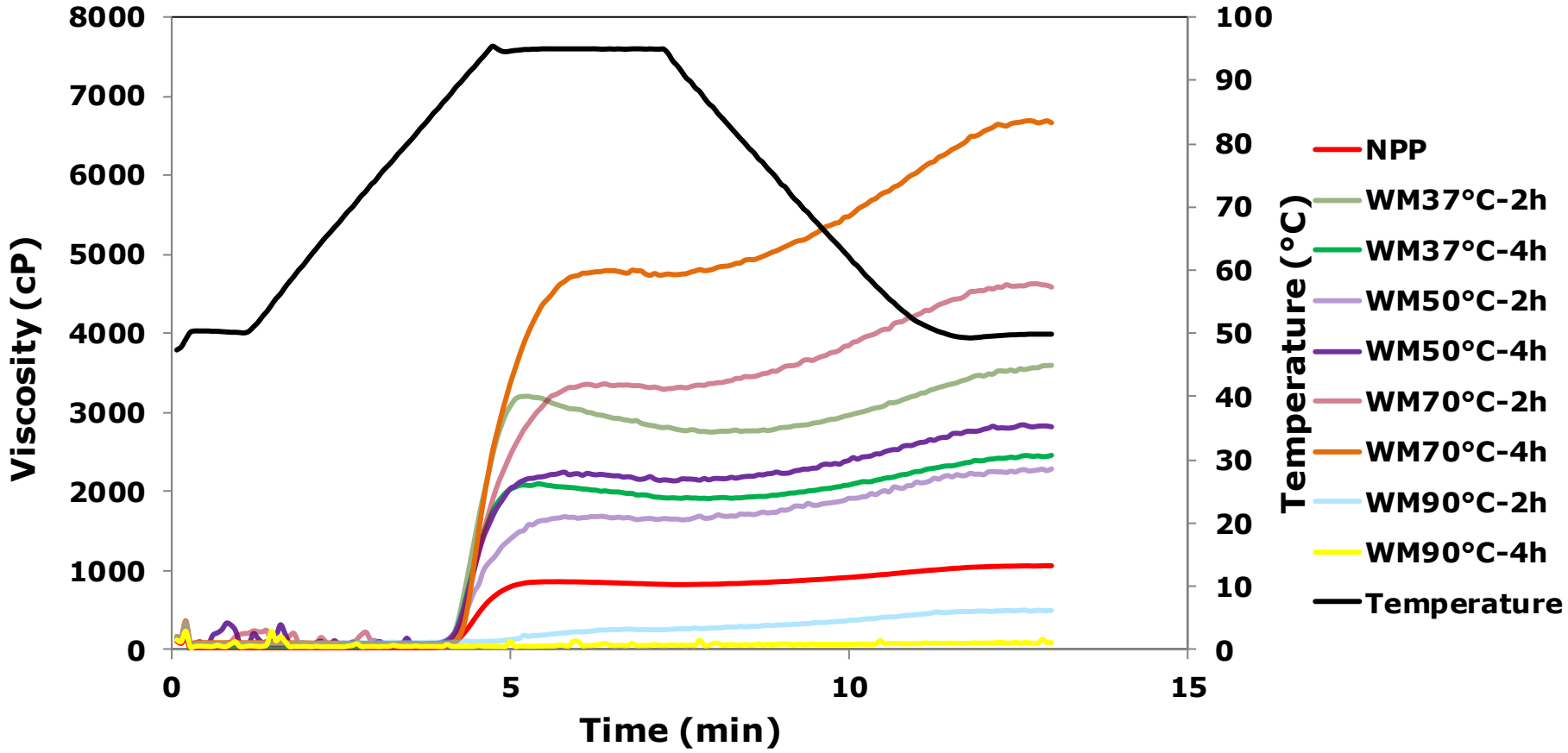



**Figure 5-9:** Pasting profile of HTT seeds after wet milling.

Possibly due to increase in heat transfer during hydration at 70°C-4h and mechanical disruption of cellular structures at wet milling, there was much increase in peak viscosity though no cold water viscosity. The figure legend indicates soaking temperature (°C) and time in hour (h) while WM indicates wet milled. NPP: native non-treated pigeon pea.

The major feature of the curves in Figure 5-9 showing the viscosity and therefore, the swelling behaviour is the absence of any major cold water viscosity at the initial pasting temperature (50 °C) within the RVA by all the wet milled samples. Since the HTT at 37-70 °C was carried out at a temperature lower than pigeon pea starch gelatinisation, melting of crystalline structure was expected not to occur at 50 °C and hence the starch would not be expected to absorb water and swell to cause a measurable cold viscosity in the RVA. The peak viscosity of the wet milled samples especially WM70°C -4hin Table 5-2 and Figure 5-9 showed several-fold increase (4821.33) in peak viscosity when compared to NPP (663 cP). This indicated that the peak viscosity was not dominated by the starch properties alone, as these samples would all have native starch, but could be the consequence of changes to the cellular structure during HTT. The low swelling of the native samples compared to the hydrated samples shows that the material cannot swell much, and that subsequent hydration makes it easier for the particles to swell. The degradation of the cell wall materials (middle lamella) during the initial long soaking time (4 h), high temperature (70 °C) and wet milling before pasting could contribute to the continuous wetting of the both the hydrophobic and hydrophilic polymers' surfaces. This may aid the binding of the materials with water and thus enhanced water availability, reduction to cellular wall rigidity and these factors could all facilitate the swelling of granules to enhance the overall hydrodynamic volume of the system at the elevated temperatures (Dhital et al., 2016).

Surprisingly the peak viscosity of WM37°C-2h, WM37°C-4h, WM50°C-2h and WM50°C-4h differed significantly, even when they had similar equilibrium moisture contents and seed textural deformation forces. This implies that the seeds may acquire similar moisture content and hardness during hydrothermal treatment, but different structural events occur. Therefore, the use of deformation force as an indication of softening of the

seed is not by itself an indicator of how the seed may perform of cooking in excess water.

Except the wet milled at 90 °C, all other wet milled samples (37-70 °C) final viscosity was observed to be higher than NPP. Presumably, due to the pre-treatment and loss of kinetics during cooling, greater solubilisation of gelatinised starch occurred into the pasting solution.

#### 5.2.5.3 Pasting properties of 37-90 °C HTT after dry milling

The resultant powders from samples created from dried and milled HTT (section 3.2.3) were evaluated using the RVA and all the pasting viscosities are shown in Figure 5-10 and Table 5-2.

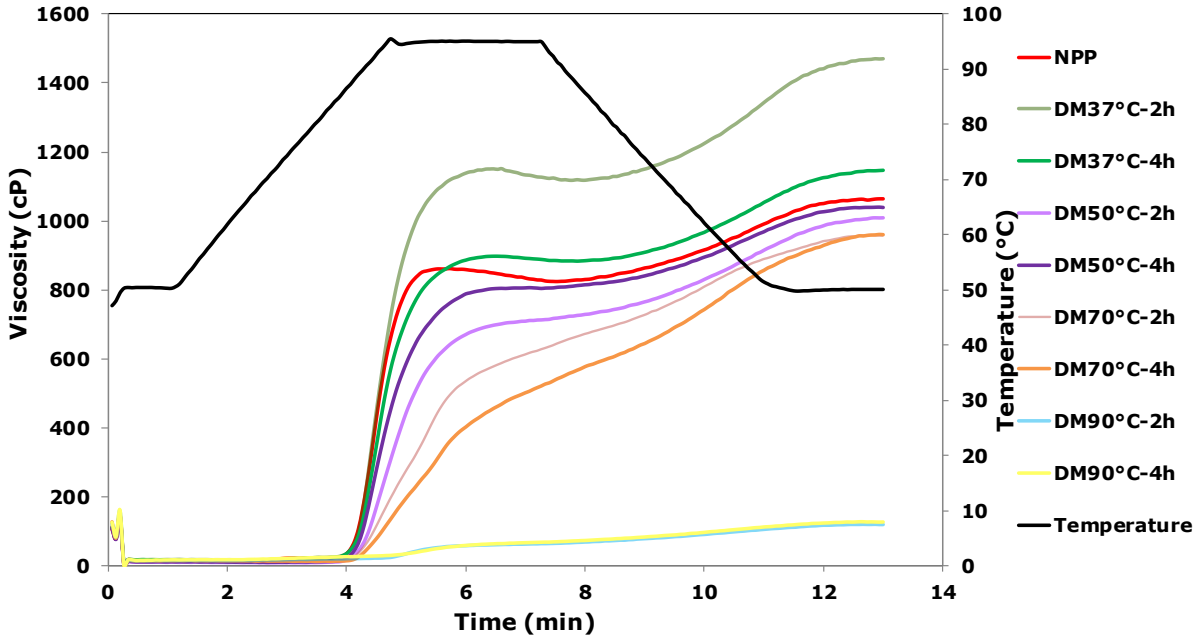



**Figure 5-10:** Pasting profile of HTT seeds after dry milling.

The figure legend indicates soaking temperature (°C) and time in hour (h) while DM indicates dry milled. NPP: milled powder from native (non-treated) seed.

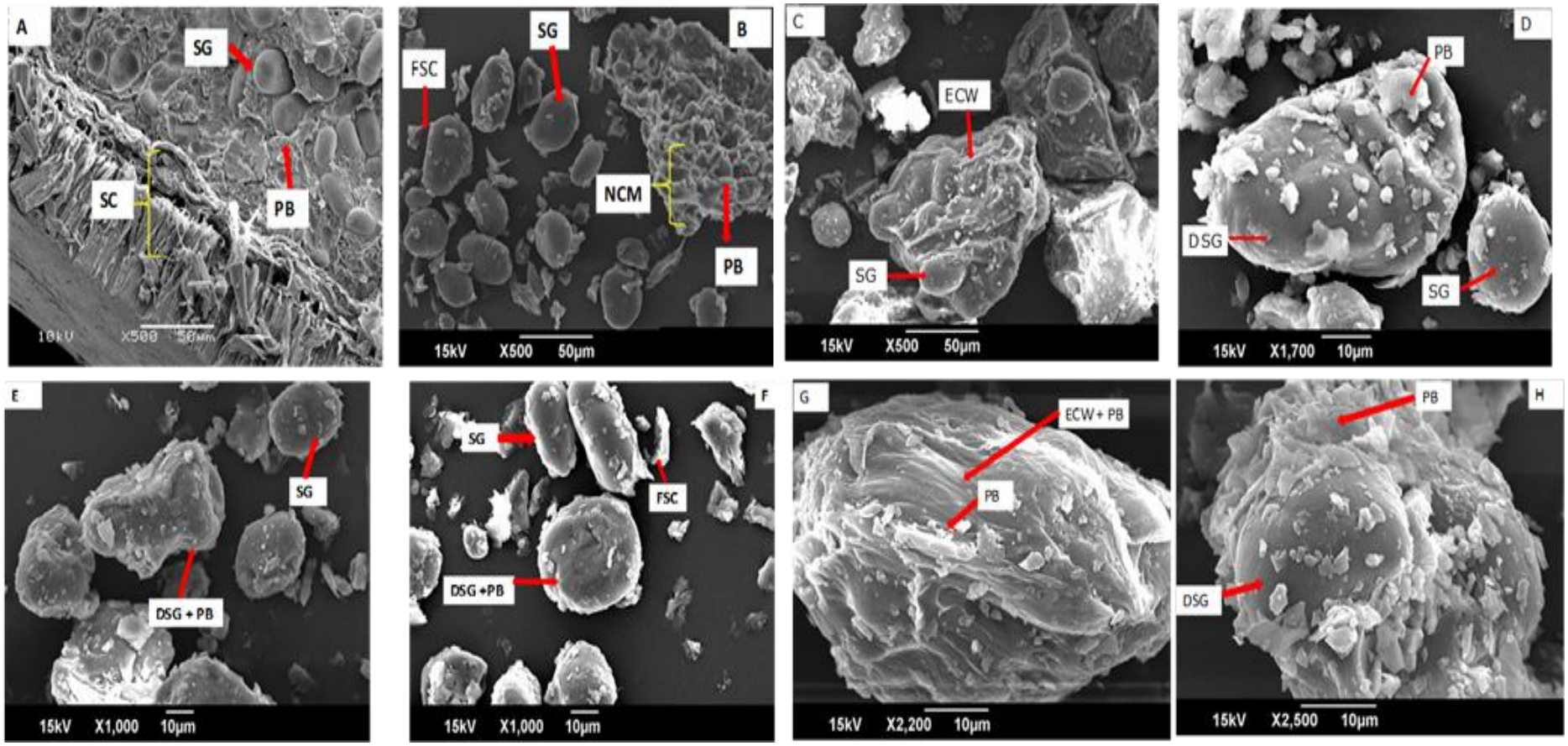
The pasting viscosities of all dry milled samples values obtained were lower than the wet milled samples, even though the solids concentration were calculated to be the same. Except DM37 samples, all other dry milled samples had a lower peak viscosity when compared to NPP. Between the

37-70 °C dry milled samples, the peak viscosity value was without significant difference at $P<0.05$. The loss of moisture caused by the long period of drying the high moisture (52-61%) soaked samples together with the dry milling must have re-structured the material's hydrated matrix to a form that is more difficult to absorb water. It is important to note that samples that have undergone HTT will lose the benefits of the treatment if dried afterwards.

### 5.2.6 Microstructure of wet and dry milled 37-90 °C HTT seed flour

To establish how the thermal treatment in addition to the wet and dry milling influenced the native morphology (protein bodies, starch granule, seed coat and cell wall) of the samples, microscopic images were examined using a Scanning Electron Microscope (JEOL JSM-6490LV, Tokyo, Japan).

**Figure 5-11**A-G: microstructure of wet and dry milled HTT.




(A) Native matrix of pigeon pea seed from the seed coat cell layers to the embedded starch granule on the protein matrix (B) Native starch disrupted during milling & separate protein bodies (C) soaked at 37 °C and wet milled (D) Soaked at 37 °C and dry milled (E) soaked at 50 °C and wet milled (F) Soaked at 50 °C and dry milled (G) Soaked at 70 °C and wet milled (H) Soaked at 70 °C and dry milled. PB: protein bodies, SG: starch granule, DSG: deformed starch granule, ECW: elongated cell wall, NCM: native cell matrix, FSC: fragmented seed coat, SSG: swollen starch granule, SC: seed coat layers of cells.

The images of the microstructure Figure 5-11shows that the native starch granule smooth surface was affected during the various processing (hydrothermal treatment and milling). The cellular surface structures of wet and dry milled samples soaked at 70 °C for 4 hours (Fig G and H) showed that both HTT and the different milling processes caused changes in the native cell matrix (NCM) and starch granule. An intact starch granule with deformed surface was observed in Figure D, F and H, where the samples were soaked at 37 °C, 50 °C and 70 °C and dehydrated in vacuum dryer at40 °C. It is also possible that dry milling of the dehydrated samples reduced the rigidity of the cell wall and enhanced the disruption of the starch from the native matrix where the granule was initially embedded on the protein matrix. In addition, the disruption from the milling process enhanced cell separation and deformation of the intact starch granule. As the HTT temperature increased to 50 °C (Figure 5-11E) and 70 °C (Figure 5-11G), the cell wall fibrous materials elongated and completely enclosed all the cotyledon cellular components (starch granule and protein bodies) into a continuous matrix. From Figure 5-11 D-G, some white particles, most likely to be the protein bodies from the milled cell, attached themselves onto the disrupted and deformed starch granules.

Although the DSC could not ascertain a decrease in either gelatinisation enthalpy or temperature (peak and endset) of the 37-70 °C HTT samples, when compared to the untreated control (Figure 5-11A-B), Figure 5-11G shows that the soaking at 70 °C and wet milling led to the formation of a more continuous network. This continuous network could be attributed to the solubilisation of middle lamella of the cell wall and may have resulted in increased accessibility of water into the cells, hydration of starch and the subsequent high pasting peak viscosity of 70 °C wet milled samples. The appearance of protein bodies surrounding the starch granules in some areas of the milled cellular structure (Figure 5.11 A-H) is an evidence of strong starch-protein matrix in pigeon pea seed though dry milling

facilitated their separation (Figure 5.11A, B, D and F). However, their separation did not improve the DSC gelatinisation properties.

### 5.2.7 Conclusion on the impact of HTT at 37 °C-90 °C

A consequence of the rapid uptake of water, particularly by the seed coat (through diffusion actions), is the softening and wrinkling of the seed coat which occurred at 30 min of soaking. Although the equilibrium moisture content of the HTT samples was relatively the same with minimal impact of temperature, a higher soaking temperature of 70 °C or 90 °C, decreased the equilibrium time by over twofold when compared to the time required for low soaking temperatures of 37 °C and 50 °C. Based on the optimal conditions surrounding the equilibrium moisture content of the present hydration method, an optimised ambient soaking condition (37 °C, 150 min) was selected, which is appropriate for the seed to reach the equilibrium moisture content of 60% compared to the conventional 12 hours soaking. It is proposed that reduction of seed hardness when processed at 37 to 70 °C resulted from the cell separation following water uptake into the interstitial spaces and softening of middle lamella. This contrasts with seed softening and reduction in seed hardness after starch gelatinisation at 90 °C HTT.

The rupture of the native cell matrix during wet milling and the equilibration of moisture after wet milling enhanced the material pasting properties. Presumably this was due to changes in the cell wall and middle lamella, as limited structural and thermal changes had occurred in the starch granule during soaking at 37 to 70 °C. An indication of the limited changes in granular gelatinisation properties at 37 to 70 °C HTT were detected through the DSC thermal analysis. These samples indicated higher on-set gelatinisation temperatures due to perfection of the crystals structures from annealing compared to the native un-treated sample. A confirmation that complete solubilisation of the cellular components and disruption of starch

crystalline structure which may have occurred at 90 °C-4 h is shown by the absence of gelatinisation enthalpy, cold water and peak pasting viscosity of the wet and dry milled samples. This may suggest that the retrogradation of the gelatinised amorphous starch can occur after 90 °C-4 h.

Hydrothermal treatment, below the conditions required for starch gelatinisation, would seem to enhance the ability of wet milled samples to hydrate and swell in excess water once the particles were exposed to a second processing temperature within the starch gelatinisation temperature range. However, this ability would seem to be lost if the treated samples are again oven dried. Hydrothermal treatment at low temperature can be carried out and there would be advantages with the reduced energy requirement during cooking if the final cooking treatment occurs immediately before the final cooking stage at starch gelatinisation temperature so that the starch retains its crystallinity.
Moisture uptake and the hardness of the pre-treated seed are not direct predictors of starch thermal properties and how the starch will interact with excess water. The results obtained from the HTT at 37 to 90 °C indicate that disruption of cellular organisation and structural components of the pigeon pea are important for hydration and cooking of pigeon pea seed. Although time and temperature may assist in the softening of the pigeon pea, other processes targeting the degradation of the cell walls may be more effective pre-treatment.

## 5.3 Seed softening targeting cell wall degradation

The impact of short time (5 min) hydrothermal (steaming)with or without alkaline and enzymatic (application of cell wall degrading enzyme) treatment in enhancing hydration and softening of the seeds through degradation of the cell walls are discussed in this section. One of the aim of this treatment was to soften the seed using short time steam treatment targeting degradation of pectic substances (beta elimination of pectin at

high temperature) of the cell walls and middle lamella .The technique can is further based on ion exchange displacement reaction between monovalent cation of the alkaline solution and magnesium and calcium from the middle lamella (Del Valle et al., 1992b, Kinyanjui et al., 2015, Dhital et al., 2016). The enzymatic treatment was applied to ascertain if optimal condition of the application of cell wall degrading enzymes will quicken the cell wall degradation. A stirred jacketed vessel was used for these treatments as this was thought to enhance homogenous distribution of heat. In general, it is expected that these treatments will increase starch accessibility to water and quicken gelatinisation since most of the cell wall material that has high affinity to water (water binding polysaccharides) will be solubilised. The results from these treatments will be considered in the selection of the most appropriate flour for extrusion cooking.

The hydrothermal-steam (tap water) treatment was carried out at 90 °C for 5 min with native white Agatu pigeon pea seed using a stirred jacketed process vessel (Roboqbo). The alkaline treatment was conducted in the same way as the hydrothermal- steam treatment except that tap water was replaced with 0.5% sodium bicarbonate solution at pH 8.5. The enzymatic treated samples were prepared by treating wet milled samples from the hydrothermal- steam treatment and alkaline treatments with 0.5 % (w/w) viscozyme L, containing a wide range of carbohydrase. While the enzymatic treated native whole flour serves as the control. The methods are available in section 3.2.2.4. After freeze drying and milling the treated samples, the dry powder obtained were analysed for their hydration characteristics (water absorption index, cold-water solubility, swelling factor), pasting properties, thermal properties and microstructures (see chapter 3). An isolated starch from native pigeon pea was used to ascertain the extent of the cell wall degradation treatment on hydration and thermal characteristics of the treated samples. The sample codes after the treatment are found in Table 5-3.

**Table 5-3:**Sample abbreviation after cell wall degradation

| Sample code | Description |
|---|---|
| NPP | Native pigeon pea |
| NPPS | Starch from native pigeon pea starch |
| HTT | Hydrothermal-steam treated seed at 90 °C for 5min |
| BB | Alkaline treatment at 90 °C for 5min |
| NPPZ | NPP+ enzymatic treatment |
| HTTZ | HTT+ enzymatic treatment |
| BBZ | BB+ enzymatic treatment |

### 5.3.1 Re-hydration characteristics in excess water following hydrothermal steam-alkaline-enzymatic treatment

The behaviour of flour from freeze dried treated samples (hydrothermal-steam, alkaline and enzymatic) in excess water were evaluated through the determination of hydration characteristics (swelling factor, water absorption and solubility index). The results are shown in Table **5-4**. All the results were significantly different ($p<0.05$). Through the determination of the hydration characteristics, the powders-particle interactions with water at different conditions was evaluated, especially the extent of disruption of starch from the native matrix.

**Table 5-4:** Hydration characteristics after cell wall degradation treatment.

| Sample | Swelling power (g/g) | Water absorption index (g/g) | Cold-Water solubility (%) |
|---|---|---|---|
| NPP | 5.67±0.22$^{a}$ | 3.75±0.08$^{c}$ | 13.36±0.12$^{b}$ |
| NPPS | 10.79±0.12$^{d}$ | 1.20±0.04$^{a}$ | 0.12±0.14$^{a}$ |
| HTT | 6.29±0.00$^{ab}$ | 3.88±0.22$^{c}$ | 13.6±0.05$^{b}$ |
| BB | 6±0.06$^{ab}$ | 3.98±0.01$^{c}$ | 16.25±0.2$^{cd}$ |
| NPPZ | 5.84±0.19$^{ab}$ | 3.37±0.09$^{c}$ | 14.50±$^{bc}$ |
| HTTZ | 6.73±0.11$^{b}$ | 2.58±0.02$^{b}$ | 17.52±0.25$^{d}$ |
| BBZ | 7.68±0.12$^{c}$ | 2.43±0.11$^{b}$ | 23.44±0.42$^{e}$ |

Mean values are triplicate, and mean having the different letters in the same column are significantly different ($P<0.05$) as per Tukey multiple comparison calculated by difference. NPP: Native pigeon pea flour, NPPZ: NPP+ enzymatic treatment, HTT: Hydrothermally at 90 °C for 5min, HTTZ: HT+ enzymatic treatment, BB: Hydrothermally with alkaline, BBZ: BB + enzymatic treatment.

#### 5.3.1.1 Swelling power

The swelling of starchy foods (through increase in weight and volume) during cooking is an evidence of adequate hydration and good cooking quality of the starchy material. The swelling power under quiescent condition was determined by the weight of water absorbed by the starch granule and the non-starch components of the flour from hydrothermal-steam, alkaline and enzymatic treatments when heated at 90 °C in excess water.

The results in Table 5-4show that hydrothermal-steam treatment (HTT) increased the swelling power by 11% followed by combined alkaline-enzymatic treatment (BBZ). Possibly, the influence of the sodium bicarbonate used in the alkaline treatment favours the enzymatic

degradation of the cell wall materials so that swelling power of starch in BBZ can increase. If water cannot easily enter into the cells to hydrate the cell, then application of alkaline treatment will degrade the cell walls, reduce the cooking of globular protein (due to alkaline pH which increasing the denaturation of the protein) and starch will have less materials to compete for the available water. It has been reported that soaking in alkaline solution softens legume seed and makes the cooking easier through the solubilisation of the rigid middle lamella (Agunbiade, 1996). Therefore, it was expected that either alkaline treatment (BB) or in combination with enzymatic treatment (BBZ) would facilitate initial hydration, softening and swelling of the seed so that starch gelatinisation and swelling would be enhanced during this swelling power analysis. The gelatinisation of starch through strong alkaline treatment has been reported in literature (Rafiq et al., 2016). Therefore, a decrease in swelling power is the consequence of low hydration and softening of the seed during the treatment as water accessible to starch was limited or the starch is enclosed in the cell matrix.

Reports have shown through the state diagram, section 2.4.3,that the exposure of amorphous regions of the starch to water (plasticisation effect) increases mobility among the starch polymer chains within the amorphous and crystalline domain (ordered double helical structure)(Tester and Debon, 2000, Ratnayake, 2006). Since the starch crystalline structure may have been destabilised through the hydration that occurred during the cell wall degradation treatment, therefore there would be an increase in the ingress of water and swelling of the granules during the determination of the swelling power. The swelling behaviour of the starch found in non-treated flours (NPP and NPPZ) seems to be limited due to the influence of cell wall matrix thereby leading to low swelling power (5.67 g/g) in the non-treated native seed (NPP). The low swelling of NPP is in contrast to the high swelling power (10.79 g/g) of pure starch (NPPS) without native cell wall matrix.

The increased swelling behaviour after the enzymatic treatment reveals that the enzymatic treatment was more effective in improving accessibility of the starch granule to bind with more water due to the degradation of non-soluble fractions (non-starch polysaccharides) of the cell wall materials to a soluble material. This result is in contrast to the report that the swelling power of whole chickpea flour (9.2-10.4 g/g) was lower than whole flour from pea (10.8-11.8 g/g) and lentil (10.9-11.9 g/g) due to its small amount of hydrophilic carbohydrate and water binding/soluble protein (Chung et al., 2008). Compared to pigeon pea, this report suggests that in some legumes, the presence of hydrophilic carbohydrates (non-starch) and water binding protein would increase the swelling power.

Many factors could be responsible for the limited swelling of the starch when embedded in the whole seed powder matrix following cell wall degradation treatment. These factors could be similar to the proposed scenarios in section 5.2.5 which affects the swelling behaviour of starch in HTT milled seed powderduring pasting.These factors as listed includes (i) if the starch is trapped in the whole flour native cellular matrix, it will be difficult to be hydrated even when in excess water. (ii) When starch is not sufficiently hydrated, swelling will be limited. (iii) If by chance, the starch granule within the cell matrix happens to be cooked, swelling will also be limited due to small free space (volume) in the cell which would not allow starch to expand. Finally, the free starch outside the cell, as in milled flour, when hydrated can swell up to ten times in volume.

#### 5.3.1.2 Water absorption index

The water absorption index of starch based material is usually influenced by starch since it is the major component. Starch in the native form will not absorb much water due to limited exposed hydrophilic region except when cooked. Similarly, if the cooked starch is entrapped in the cell, it will be difficult to hydrate but if the cooked swollen starch is free, it will absorb more water. Table 5-4 indicates that the cold water absorption index

(measured at 20 °C room temperature) of NPP (3.75 g/g) and all the treated samples (2.43 to 3.98 g/g) were at least two-fold higher than the native starch sample (NPPS). It looks like the 6% increment in the water absorption after the hydrothermal alkaline treatment is as a result of destabilisation of the helical order in starch granular structure compared to the uncooked pure starch (NPPS) with low water absorption index. The WAI (treated and non-treated pigeon pea) obtained through centrifugation method were higher than reported dehulled pigeon pea flour (1.388 g/g) (Kaushal et al., 2012)and some whole legume flours such as lentil 1.2-19 g/g and horse gram 1.5-1.9 g/g (Ghumman et al., 2016). Apart from starch,the absorption of water by other hydrophilic polymers of pigeon pea (protein and non-starch polysaccharides) can contribute to the increase in the cold water absorption index.

Surprisingly HTTZ and BBZ with initial increase in swelling power had a 35% reduction in the water absorption index while NPPZ with low swelling power, displayed higher water absorption index. These variations in cold water absorption and swelling depends on the earlier mentioned factors that affects the physical state of starch. The enzymatic degradation of the cell wall components has been proposed to increase starch access to water and swelling during the re-heating process (determination of the swelling power) if more of the water-holding cell wall materials were degraded. But if otherwise, then the available water will still be trapped by these native hydrophilic polymers. Therefore, due to the presence of free native granule following the degradation of cell wall materials and milling, cold water would decrease because free native granule has low affinity to cold water while swelling will be favoured because the granules were free and not encapsulated.

#### 5.3.1.3 Cold water solubility

The results of the cold-water solubility in Table 5-4 suggest that treatment with alkali or enzymes contributed to more leaching of soluble material compared to the native flour (NPP). When compared to NPP, the combined alkaline and enzymatic treatment (BBZ) resulted in >50% increase in the amount of water soluble materials. This could be due to the release of more soluble materials from the cell wall after its degradation in the native seed. Even without the additional use of enzymes, the alkaline-hydrothermal treatment at pH 8.5 resulted in a 19% increase in the leaching of water-soluble materials compared to the hydrothermal treatment (HTT) carried out at neutral pH. The composition of the leachate from the alkaline treated samples was not established but hemicellulose (cell wall material) may be present since it is easily solubilised in an alkaline environment.

However, the leaching of solute in cold water occurred mostly after alkaline and enzymatic treatment (BB, BBZ and HTTZ) as most of the leached solutes were solubilised material from the degraded cell wall materials. The low cold-water solubility of HTT is evidence that degradation of pigeon pea cell wall materials was limited by hydrothermal treatment alone. As earlier stated by many authors (Mattson, 1946, Aguilera and Rivera, 1992, Waldron et al., 2003, Brummell, 2006, Kinyanjui et al., 2015), soaking or cooking of legume seed with various sodium salt solutions softens and reduces the cooking time of the seed through displacing the calcium in the insoluble pectate of the cotyledon cell middle lamella with sodium ion from the solution. Hence more leachable solutes will be available from the treated sample when dispersed in excess water. The low cold-water solubility (0.12%) of the pure native starch sample (NPPS) confirmed that majority of the leached solutes in BB and BBZ were not starch materials but non-starch polysaccharides and cell wall proteins.

### 5.3.2 Pasting characteristics after cell wall degradation

The hydration characteristics that were measured in the previous section 5.3.1 were re-assessed during a heating cycle in Rapid Visco Analyser with 12.5% (w/w) solid content of the sample powders suspended in water (full method in Chapter 3 section 3.7.1). With the RVA result, the changes initiated by the treatment which affects the integrity of starch granule may be understood. The numerical data of the pasting properties is in Table 5-5 alongside the pasting profiles of treated samples in Figure 5-12 and the isolated starch in Figure 5-13. Compared to the native non-treated flour (NPP) with 647 cP peak viscosity, the treatment enhanced the peak viscosity (895-1297 cP). It appears as if the non-treated cell wall materials present in NPP restricted the starch from swelling. Notwithstanding the high treatment temperature (90 °C), none of the treated samples showed a cold water swelling peak, which could be expected if the sample had contained pre-gelatinised starch. Possibly the short treatment time (5 min) could not allow changes in the starch crystalline structure.

(Refer to section 3.8 for features of the pasting curve)

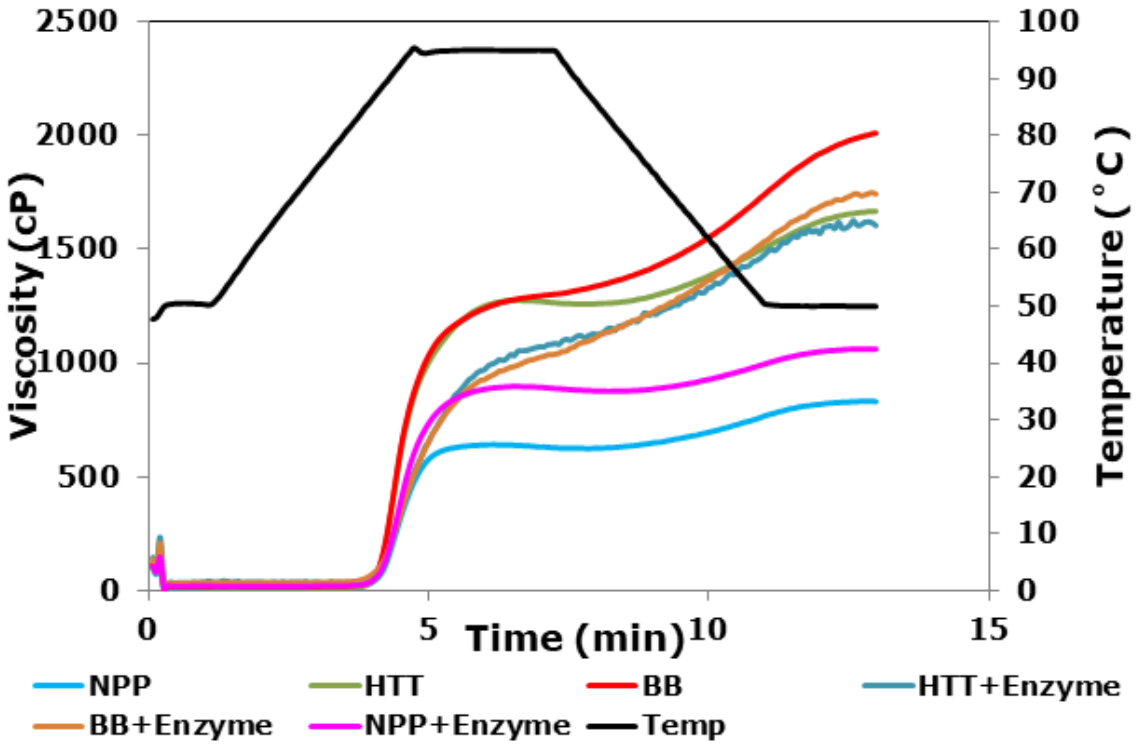



**Figure 5-12:** RVA pasting profile of 12.5% (w/w) solid pigeon pea powder after hydrothermal, alkaline and enzymatic treatments.

NPP: Native pigeon pea flour, NPPZ: NPP+ enzymatic treatment, HTT: Hydrothermally at 90°C for 5min, HTTZ: HT+ enzymatic treatment, BB: Hydrothermally with alkaline, BBZ: BB + enzymatic treatment, NPPS: Isolated starch from native pigeon pea seed.

**Table 5-5:** RVA pasting properties after cell wall degradation.

| Sample | Peak viscosity (cP) | Final viscosity (cP) | Setback(cP) |
|---|---|---|---|
| NPP | 647±15.59$^{a}$ | 834.33±18.88$^{a}$ | 221.58±40.65$^{a}$ |
| HTT | 1281±43.30$^{c}$ | 1670.66±29.28$^{b}$ | 417±21.79$^{b}$ |
| BB | 1297.33±33.86$^{c}$ | 2014±37.47$^{c}$ | 765.33±13.48$^{cd}$ |
| HTTZ | 1076.33±61.83$^{bc}$ | 1605.33±86.12$^{b}$ | 616.33±16.80$^{c}$ |
| BBZ | 1023.66±22.68$^{bc}$ | 1741.33±53.46$^{b}$ | 807.33±42.15$^{d}$ |
| NPPZ | 895±61.83$^{b}$ | 1059±62.64$^{a}$ | 186±6.24$^{a}$ |
| NPPS | 9722±99.86$^{d}$ | 7338±10.76$^{c}$ | 1056±13.66$^{e}$ |

Mean values are triplicate and mean having the different letters in the same column are significantly different ($P<0.05$) as per Tukey multiple comparison calculated by difference. NPP: Native pigeon pea flour, NPPZ: NPP+ enzymatic treatment, HTT: Hydrothermally at 90°C for 5min, HTTZ: HT+ enzymatic treatment, BB: Hydrothermally with alkaline, BBZ: BB + enzymatic treatment, NPPS: Isolated starch from native pigeon pea seed.

Bearing in mind that RVA is a heating treatment in its own right, the peak viscosities in Table 5-5were higher in hydrothermally (HTT) and alkaline treated samples with or without enzymes. Notwithstanding that, the swelling power of HTT was low and similar to NPP, its peak viscosity was two-fold higher than NPP. It was initially thought that the low swelling powers of these samples were as a result of low hydration of starch but the display of high RVA peak viscosity is an indication that other structural transformations must have taken place in the sample which are obvious when the samples is sheared and heated during pasting. This may be the impact of the second scenario of starch after the treatment (section 5.2.5) whereby the granule having been hydrated could not gelatinise at the appropriate temperature due to the limited mechanical shear. The difference in the procedure used for measuring swelling power and peak viscosity, affects both the water absorption and swelling of the starch

granule. The swelling power was conducted in absence of shearing with 1% solid concentration within steady high temperature (90 °C) while the pasting peak viscosity was determined under shearing with 12% solid concentration and could cause a higher heterogeneity of particle behaviours as the sample is heated from 50-85 °C (Parker and Ring, 2001). In addition, the disruption of the granule from the cellular protein matrix during the pasting high shear treatment, enabled the exposed granule to have more access to water during the pasting-heating process thereby increasing the peak viscosity (Dhital et al., 2016). Depending on the extent of interactions of the gelatinised starch with the denatured protein, the peak viscosity would be affected (Huang et al., 2007, Kaur and Sandhu, 2010a, Kaur and Sandhu, 2010b, Acevedo et al., 2013).

The increase in peak viscosity of BB and HTT after the enzymatic degradation treatment could be due to the detachment of the granule (free granules) from the intact cell walls and subsequent increase in hydration, and swelling. This also relates to the reason for the high swelling power of these samples even under a shear less condition. With their decrease in water absorption and high cold water solubility, this treatment will be selected as most appropriate method for degradation of cell wall materials of pigeon pea.

The viscosities formed during the cooling of the paste were monitored using two parameters namely setback and final viscosity. The setback, which is a measure of recovery of viscosity after the breakdown, suggested an increase in viscosity during the cooling period as the temperature is reduced. Higher setback viscosity (417 cP-807 cP) were recorded in all the treated samples (HTT, BB, HTTZ and BBZ) except in NPP and NPPZ with low values of 221.58 cP and 186 cP respectively. This low setback viscosity observed in these samples is due to their initial low peak viscosity, which indicated some restriction on the swelling of granule. It has been reported that lower amount or absence of swollen starch granule fragment (due to

the restricted swelling of granule) embedded in the leached amylose decreased the setback viscosity (Chung et al., 2008) Therefore, higher concentration of swollen fragmented granules in the dispersed phase of the paste increased the setback viscosity of BB, HTT, HTTZ and BBZ as it is easier to form hydrogen bonds on cooling with the fragmented swollen granule than the intact swollen granule of NPP and NPPZ.

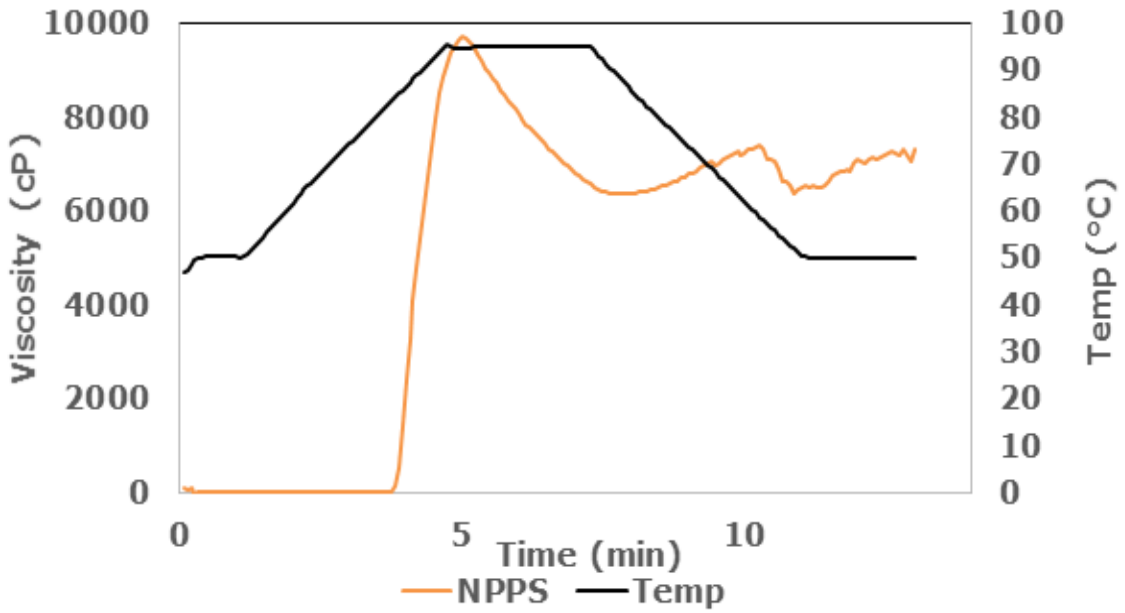



**Figure 5-13:** RVA pasting profile of 12.5% (w/w) solid pigeon pea starch (NPPS).

Compared to the pasting profile of whole pigeon pea, the isolated pigeon pea starch shows sharp peak viscosity at the beginning of holding temperature (95 °C) and high breakdown after the peak. Compared with 9722 cP peak viscosity achieved for the 100% native starch (NPPS) in Figure 5-13, all samples from the cell wall degradation treatment had a lower peak viscosity ranging from 895 cP in NPPZ to 1297 cP in BB. Confirming that a free starch granule (not entrapped in the native cell matrix) when cooked can swell ten times the initial volume. Although the pure starch (NPPS) had higher concentration of starch which is two-fold higher than the starch concentration in the treated samples, the native starch still showed much greater swelling than the whole milled powder

samples due to the exposure from encapsulated cell matrix. Hence, the more swollen the free starch granule is, the greater the tendency to breakdown and rupture on application of shear. Reference to the increased peak viscosity (3377-4821 cP) of 2 and 4 hours 70 °C wet milled samples in section 5.2.4 reveals that elimination of cell wall barrier through thermal and mechanical degradation enhances starch swelling and increases peak viscosity. Therefore, the prolonged cooking of pigeon pea (long period before starch gelatinisation) could be attributed to the entrapment of the starch granule in the native cell matrix where protein bodies and cell wall materials limits the hydration and swelling of the granule.

### 5.3.3 Characteristics of the crystalline structure following cell wall degradation treatment

During the treatments involving hydrothermal, alkali and enzymes, the pigeon pea seeds were soaked in excess water and then stirred and heated at 90 °C for 5 min. Following these treatments, it was expected that the centre of the seeds would reach temperature more than 80 °C but the limited ingress of water into the inner cotyledon cellular components only allows low moisture content (22%) to be attained. At this level of hydration, it could be expected that no or low levels of loss of starch molecular order would occur. A report on a similar thermal (100 °C) alkaline treated pigeon pea seed confirms partial gelatinisation of starch (Roskhrua et al., 2014). Therefore, the question is whether, under the unusual hydration behaviour discussed in the previous sections due to the construction of the pigeon pea matrix, the starch retained much of its order? To estimate the loss of starch molecular order, the powders created from the treatments were investigated with wide-angle X-ray diffraction and DSC.

#### 5.3.3.1 DSC thermal properties following cell wall degradation

The results of the DSC analysis presented as DSC thermogram in Figure **5-14** and numerical values in Table 5-6are used for the discussions of the impact of the cell wall degradation treatments on starch thermal

properties. Compared to the native untreated pigeon pea (NPP), there was no significant difference at the level of $P<0.05$ in the melting enthalpies (ranged from 5.5 to 7.09 J/g) of the flour due to the cell wall degradation treatment as shown in Table 5-6. There was however, a significant difference in the gelatinisation enthalpy of the pure starch (13.81 J/g) sample (NPPS) compared to treated samples. The high gelatinisation enthalpy required to melt the granule in the pure starch was attributed to high concentrations of the starch and the thermal stability of pigeon pea starch crystallites. Enthalpy in regard to starch has been defined as the amount of energy required to convert the crystalline structure in the native starch to the amorphous structure and reflects the degree of crystallinity. While, crystallite transition is an important factor controlling starch end usages, pigeon starch granule has been characterised by the close dense packing of the crystallite (high structural stability) which limits accessibility of water into the granule interior hence restricting gelatinisation (Singh et al., 2008a). Previous evaluation in Table 4-1 showed a high specific density of the seed.

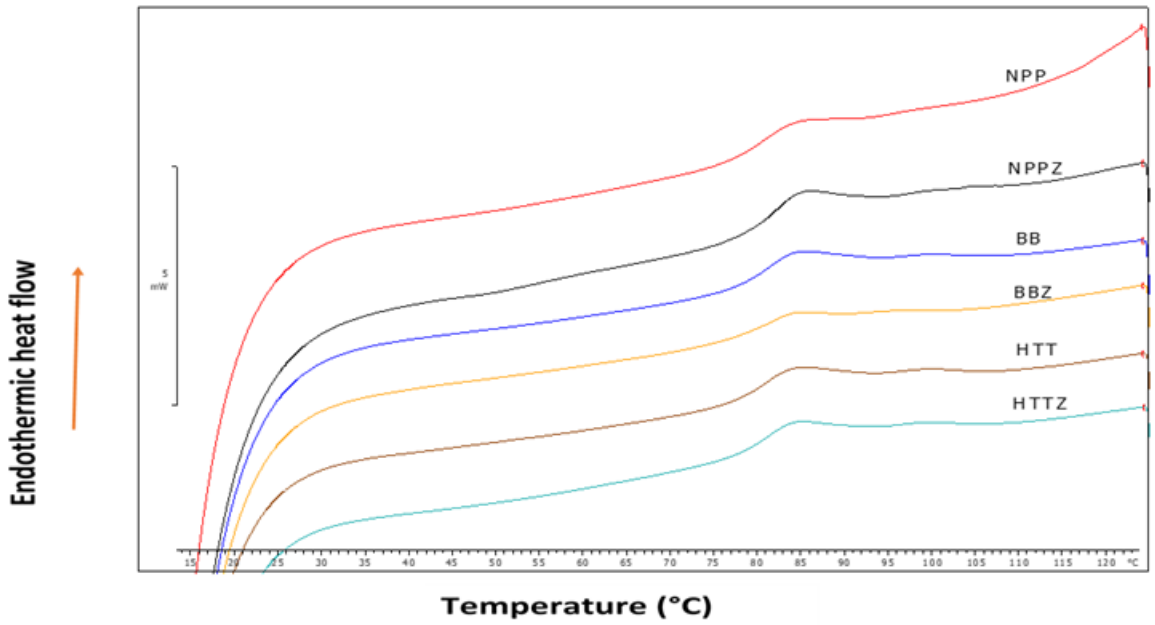



**Figure 5-14:**DSC profile after cell wall degradation treatment.

No changes were detected in the thermal properties as a result of cell wall degradation treatment.
NPP: Native pigeon pea flour, NPPZ: NPP+ enzymatic treatment, HTT: Hydrothermally at 90°C for 5min, HTTZ: HT+ enzymatic treatment, BB: Hydrothermally with alkaline, BBZ: BB + enzymatic treatment.

**Table 5-6:** Thermal properties after cell wall degradation treatment.

| Sample | Onset (°C) | Peak (°C) | Endset (°C) | Gelatinisation enthalpy (J/g) |
|---|---|---|---|---|
| NPP | 76.41±0.04$^{b}$ | 84.54±0.02$^{b}$ | 99.68±0.64$^{b}$ | 5.40±0.46$^{a}$ |
| NPPS | 72.19±1.31$^{a}$ | 78.66±0.91$^{a}$ | 91.60±3.31$^{a}$ | 13.75±0.08$^{b}$ |
| NPPZ | 77.38±0.05$^{b}$ | 84.68±0.02$^{b}$ | 98.57±0.78$^{b}$ | 6.55±0.54$^{a}$ |
| HTT | 76.09±0.33$^{b}$ | 83.97±0.49$^{b}$ | 97.86±0.24$^{b}$ | 6.66±0.63$^{a}$ |
| HTTZ | 76.87±0.04$^{b}$ | 84.68±0.07$^{b}$ | 99.59±0.42$^{b}$ | 6.67±0.20$^{a}$ |
| BB | 75.98±0.51$^{b}$ | 84.31±0.33$^{b}$ | 99.29±1.14$^{b}$ | 6.16±0.45$^{a}$ |
| BBZ | 75.97±1.51$^{b}$ | 84.21±0.91$^{b}$ | 96.01±4.48$^{b}$ | 7.09±0.19$^{a}$ |

Data are mean value of the duplicate analysis± standard deviation. Means with different letter in the same column differ significantly ($p<0.05$) with Tukey *post hoc* test**.** NPP: Native pigeon pea flour, NPPZ: NPP+ enzymatic treatment, HTT: Hydrothermally at 90°C for 5min, HTTZ: HT+ enzymatic treatment, BB: Hydrothermally with alkaline, BBZ: BB + enzymatic treatment. NPPS: Isolated pigeon pea starch.

Several effects of heat moisture treatment on starch crystalline structures have been reported. Similar to the present research, a study by Hoover et al. (1993) reported that non-alteration of the gelatinisation enthalpy occurred after heat moisture treatment of pigeon pea (prepared by heating starch of 30% moisture at 100 °C in an air oven for 16 h).In another study, it was reported that the enthalpy required for melting of corn and potato starches decreased following heat moisture treatment at 120 °C (Takaya et al., 2000) and 100 °C (Gunaratne and Hoover, 2002)respectively. With these reports, the listed starch scenarios in section 5.2.5 was considered to have contributed to the non-significant difference in the gelatinisation enthalpy of treated pigeon pea. Especially, the inability of the granule to be hydrated sufficiently during the treatment even when the temperature was sufficient to cause melting of the crystalline component. Probably the short

time (5 min) used for hydrothermal treatment (with or without sodium bicarbonate) was not adequate for the hydration of the starch and subsequent gelatinisation. The architecture of pigeon pea starch granule within the cells may also restrict the transformation of the granule even when hydrated.

Notably, starch transitions have a complex dependence on the water mediated melting of crystallites (Garcia and Lajolo, 1994) which affects the overall energy (measured as the enthalpy required to melt the crystallites). Therefore, the reason for the high levels of energy required to cook pigeon pea and the consequent hard to cook defects may be related to the starch characteristics as well as the cellular architecture of the cotyledon. Previous researchers associated the high gelatinisation enthalpies of legume starches with many factors. These factors include(i) Starch access to water. (ii)The presence of non-starch components (the tight association of proteins with starch can hinder separation of protein-cell wall matrix during cooking). (iii) Starch embedded in the native seed matrix (isolated free starch granule is more accessible to water than when embedded in the matrix of other seed components). (iv) Cell wall materials that encapsulate the legume starches and restricts their access to water (HTC legumes bind less to water). Therefore, with these factors, it is hard for the starch to lose molecular order(Hincks and Stanley, 1987, Garcia-Vela and Stanley, 1989, Gidley et al., 1993, Cunin et al., 1995, Acevedo et al., 2013).

It was expected that the measured gelatinisation temperatures, especially the onset gelatinisation temperature, would be higher in the processed samples as the easier to melt granules would have been melted and remaining crystals would be annealed. However, no significant difference occurred in the gelatinisation temperature compared to the native flour which indicates that the treatments did not have any effect on the gelatinisation temperature. The only difference in the gelatinisation temperature occurred on the pure starch sample (NPPS). The NPPS had lower gelatinisation temperatures compared to the whole flour samples.

Hasjim et al., (2013) observed a higher gelatinisation temperature in rice flour compared to the isolated starch. The high gelatinisation temperature in rice flour was attributed to the presence of non-starch components (protein and cell wall materials) which hinders water diffusion and penetration into the granule thereby increasing the temperature required to melt the granule with limited water. Similar to legume starches, high gelatinisation temperature range (73-101 °C) was observed in all the samples irrespective of treatment (Garcia-Vela and Stanley, 1989, Garcia and Lajolo, 1994).

Since the treatment did not alter the gelatinisation enthalpy of the treated samples compared to the native pigeon pea samples, then a conclusion could be that the thermo-stability of pigeon pea starch granule contributed to the hard to cook defect that hinders seed softening and increased the cooking time. Therefore, a severe shearing treatment such as in extrusion would be required to destabilise the crystallite structure of pigeon pea starch and possibly transform the material into a convenient product rather than using the hydrothermal treatment for increased utilisation of pigeon pea.

### 5.3.3.2 X-ray diffraction and relative crystallinity

To investigate the type of crystallinity occurring in the pigeon pea, a wide angle x-ray diffraction pattern was obtained according to the method in section 3.8.1. Compared to the native flour, a change in the X-ray diffraction pattern will indicate a transition in the crystalline structure, which can be used to substantiate any claim in the DSC analysis or other analytical results. The patterns of the treated and native samples are shown in Figure 5-15 while the value of the relative crystallinity is shown in Table 5-7. From Table 5-7, the relative crystallinity of the pigeon pea flour (3-6%) and native starch (14%) are lower than most legume that ranged from 17-25% (Zhou et al., 2004, Sandhu and Lim, 2008). The lower concentration of starch in the milled seed powder because of the presence of non-starch components will have lowered the crystallinity content in terms of mass ratio.

**Table 5-7:**Relative crystallinity after cell wall degradation.

| Flour | Relative Crystallinity (%) |
|---|---|
| NP | 5±0.99[a] |
| HT | 4±0.96[a] |
| BB | 5±0.22[a] |
| HTZ | 5±0.45[a] |
| BBZ | 4±0.45[a] |
| NPZ | 5±0.45[a] |
| NPPS | 13±1.76[b] |

Mean values having the different letters on the same column are significantly different (P<0.05) as per Tukey multiple comparison calculated by difference**.**

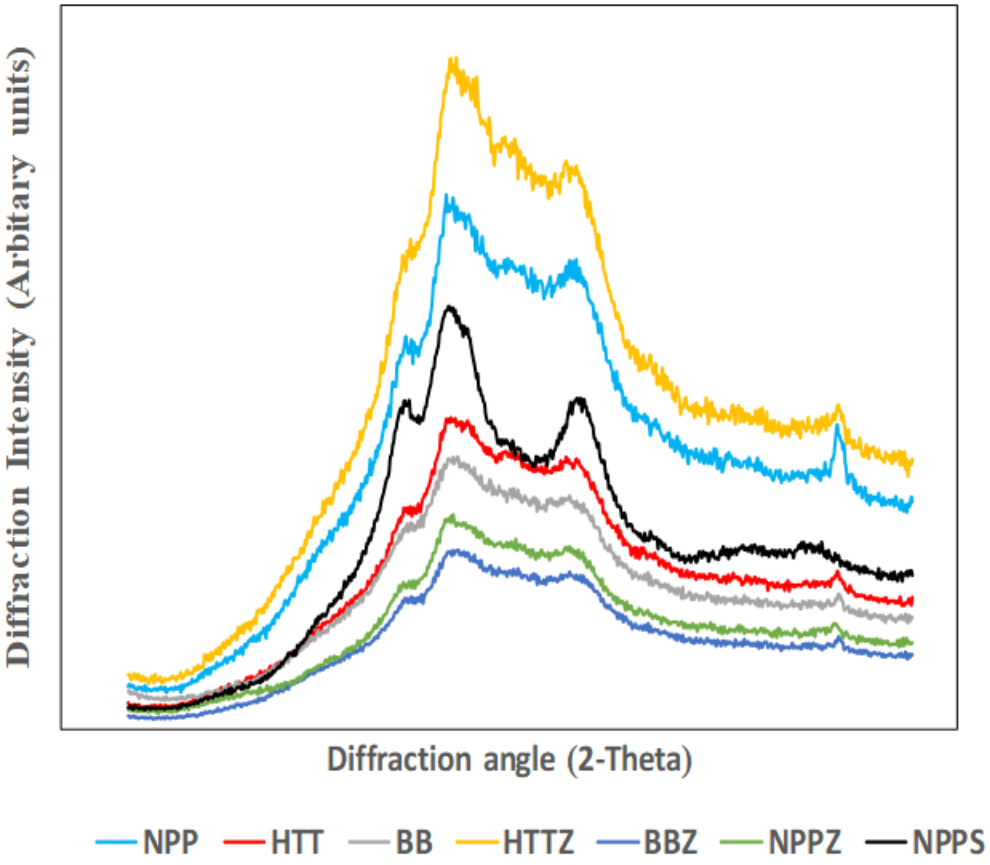



**Figure 5-15:** X-ray diffraction pattern after cell wall degradation treatment.

NPP: Native pigeon pea flour, NPPZ: NPP+ enzymatic treatment, HTT: Hydrothermally at 90°C for 5min, HTTZ: HT+ enzymatic treatment, BB: Hydrothermally with alkaline, BBZ: BB + enzymatic treatment. NPPS: Isolated pigeon pea starch.

Similar to C pattern of the native whole flour (NPP), the treated flour in Figure 5-15 gave diffraction peaks at 15.2º, 17.2º, 23.2º theta though the 15.2º seem to be broaden following the hydrothermal treatment. The C pattern of the X-ray diffraction is a combination of A-type starch (cereals) and B-type starch (tubers) X-ray diffraction pattern. (Hoover and Ratnayake, 2002, Sandhu and Lim, 2008). Surprisingly both native and treated sample had a peak at 34º 2- theta which did not appear on the isolated starch sample (NPPS) from pigeon pea. Considering that report on X-ray diffraction pattern of legumes has been mainly from pure legume starch and not from powered milled seed, hence this peak could be from

the seed globoid crystals of Phytin (phytic acid salt). Phytin as discussed as one of the anti-nutritional factor (section 2.2.2.5) are found in the protein bodies of the seed as globoid crystals structure in complex with mineral salts Possibly the complex phytate salt from the predominant minerals of calcium (120 g/100g) and magnesium (122 mg/100g) in pigeon pea ( Maenz, 2001 , Saxena et al., 2010). Most likely, more severe treatment may be required to reduce or eliminate phytin in the milled seed powder.

Except for the broadening of peak at 15.2º, loss of the long range order did not occur during the cell wall degradation treatment. Therefore, the enzymatic treatment seems to have no impact on disruption of starch crystalline structure. The high thermal stability of the pigeon pea starch crystallite may have contributed to the absence of crystalline disruption (molecular re-organisation) that did not alter the native X-ray diffraction pattern. Hoover and Manuel, (1996), reported similar non-alteration of X-ray diffraction pattern of heat-moisture treated legumes (green pea, lentil, pinto, black bean and field pea)

.

### 5.3.4 Microstructure of the cellular component following cell wall degradation treatment

The examination of the microstructure with Scanning Electron Microscope shows in Figure: **5-16** (NPP) that pigeon pea native starch granules appeared as an oval shape structure in varying sizes < 30 µm. Some of the granules were detached from the protein and cell wall matrix during milling while others are still embedded on the native matrix. The presence of non-swollen intact granules attached within the protein matrix appeared in all the images from Figure: **5-16**-through Figure -5-18. In Figure-5-17 and Figure -5-18 images, free starches were covered with the solubilised cell wall material. The scattering of the protein bodies as white patch particles all over the solubilised cells wall materials indicates the disruption of protein matrix that initially embedded the starch granule in the native matrix.

The enzymatic treatment as observed in NPPZ, HTTZ and BBZ(Figure: 5-16, Figure-5-17and Figure -5-18), led to a formation of continuous flowing mass possibly the degraded cell wall materials. However, all the treatments did not change the starch granular structure except the pasting viscosities. Hence, supporting the DSC results that the treatments did not change the crystalline structure. It has been reported that certain hydrothermal treatment which affects some physicochemical properties of the starch may not affect the granular surface (Hoover and Sosulski, 1986). As in Figure-5-17 (HTTZ) where the hydrothermally treated flour was combined with enzyme treatment, some porous structures protruded from the solubilised cell wall, due to the degradation and collapse of the cell wall. Ma et al. (2011) observed similar microstructure in some legumes flours such as lentil, chick pea and pea. The presence of solubilised cell wall (SCW) in Figure -5-18 (BB) may have resulted in the increased water absorption and peak viscosity during the pasting analysis while the decrease in the peak viscosity of BBZ and HTTZ, may be due to the higher degradation of cell wall materials resulting in low molecular weight polymers.

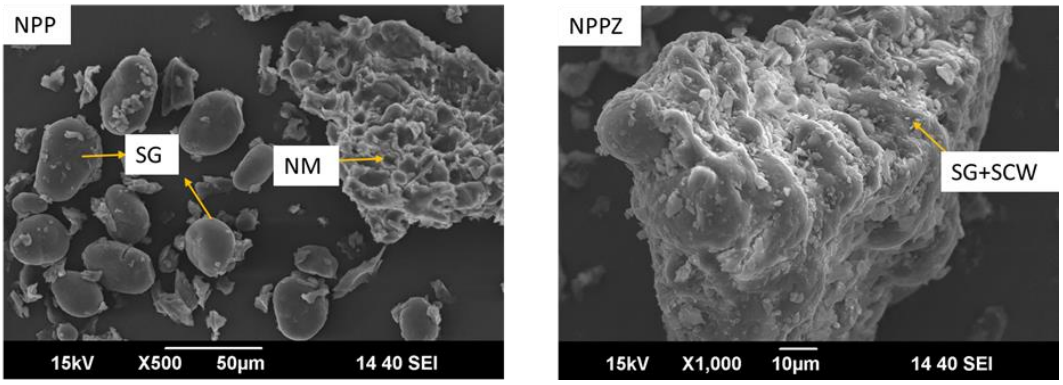



**Figure: 5-16:** NPP: Native pigeon pea flour showing starch granule (SG) protein bodies (PB) and native matrix (NM). NPPZ: NPP + enzymatic treatment.

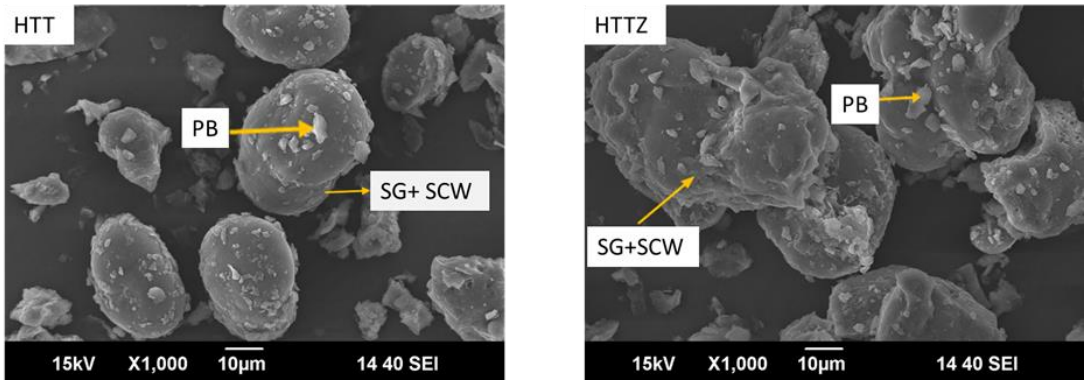



**Figure-5-17:** HTT: Hydrothermal treatment, HTTZ: HTT + enzymatic treatment.

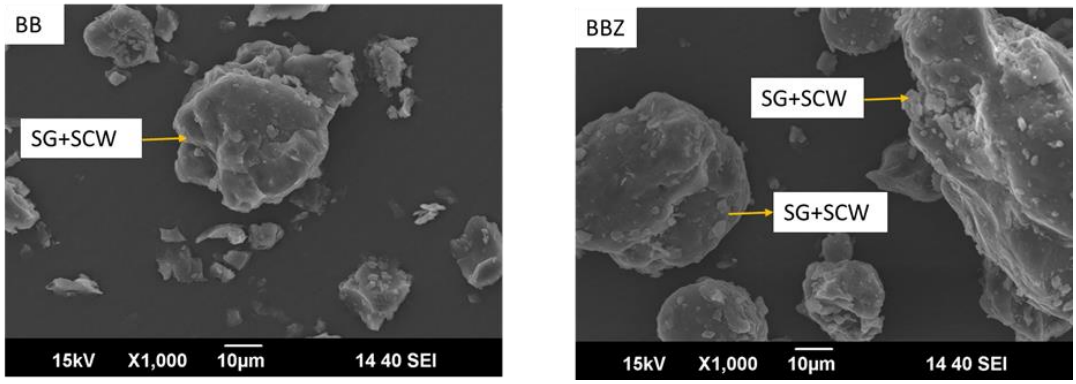



**Figure -5-18:** BB: alkaline treatment, BBZ: BB+ enzymatic treatment. SG: starch granule PB: protein bodies, SCW: solubilised cell wall, NM: native matrix.

NPP: Native pigeon pea flour, NPPZ: NPP+ enzymatic treatment, HTT: Hydrothermally at 90°C for 5min, HTTZ: HT+ enzymatic treatment, BB: Hydrothermally with alkaline, BBZ: BB + enzymatic treatment.

### 5.3.5 Conclusion on seed softening through degradation of cell wall materials

Based on results from hydration and pasting characteristics, and the microstructure, the hydrothermal treatment with or without alkali (sodium bicarbonate) and enzyme was adequate in degradation of cell wall materials. This also improved starch behaviour in excess water. No major change occurred in the starch crystalline structure except for the broadening of peak at 15.2º. The absence of major changes in starch crystalline structures may be due to limitation on the amount of water available for the facilitation of the melting of the thermo-stable crystalline structures of pigeon pea starch. Suggesting that the short time (5 min) used for hydrothermal treatment (with or without sodium bicarbonate) was not adequate in the hydration of the starch and subsequent gelatinisation. The architecture of pigeon pea starch granule within the cells may also restrict the transformation of the granule even when hydrated. Another indication of these results may be that the hard to cook defects experienced during the cooking of the pigeon pea could be due to the thermo-stable starch crystals since the starch granule could not easily gelatinised with poor swelling in the absence of shear after solubilisation of the cell wall materials.

Irrespective of the structural changes in the microstructures of pigeon pea native matrix following the cell wall degradation treatment, the DSC analysis could not detect any change in the starch thermal properties. Hence, the proposal that severe shear treatment would be best answer to destabilise the crystal structure of pigeon pea starch during processing. The proposal becomes necessary considering that the hydration of seeds at different temperatures was not adequate to destabilise the starch crystalline structure. However due to the solubilisation of the cell wall materials after the cell wall degradation treatment, this treatment in combination with appropriate extrusion conditions will applied for the proposed development of ready to eat snacks product from pigeon pea.

# 6 Development of Extruded Products

## 6.1 Introduction

Due to the limited changes that occurred in seed starch crystalline structures during the various hydration and softening treatments, as indicated in the previous chapters (chapter 4 and 5), thermomechanical extrusion cooking was considered as a processing technique. This methodology could provide major disruption of the pigeon pea structures through mechanical (high shear forces) and heat (120-150 °C) treatment. The formation of final products from extrusion cooking occurs outside the die, following the conversion of the starchy material into a viscoelastic melt and expansion, via water-steam transitions, of the melt. Considering that thermomechanical extrusion is an efficient low cost production technique used for the development of puffed and ready to eat snacks, it is expected to improve the cooking and possible utilisation of pigeon pea. Some of the relevant literature on extrusion was reviewed in section2.6.

For the thermomechanical extrusion the pilot scale TSE 24 MC Twin Screw Extruder (Thermo Scientific, Germany) was used for the development of extruded products (extrudates) from Pigeon pea. The configurations, screws and temperatures of the barrel zones used during the extrusion cooking are given in section 3.3.2, while the methodology used in the development of the extruded products is in section 3.3.3. Only the white Agatu variety of pigeon pea was selected for the thermomechanical extrusion based on availability and result of previous studies in chapter 5. Preliminary extrusion trials using milled native pigeon pea were carried out after addition of water to the feed in the extruder barrel to obtain three levels of moisture content (22%, 14% and 12%). The addition of moisture was chosen to understand the impact the different levels of plasticiser would have on extrudate formation and the change in the structural components of the legume seeds. From the preliminary findings, selections of the optimum extrusion conditions were made for further extrusion trials.

Further thermomechanical extrusion trials included the use of high moisture (22%) and low moisture (11%) powdered materials created from the 90 °C hydrothermal steam treatment and alkaline treatment in section3.3.1.The results were compared with the non-treated native pigeon pea material. In this chapter, the characteristics of the extruded products are reported and quality assessments (methodology available in chapter 3) were based on sectional expansion index (SEI), bulk density (BD), water absorption index (WAI), water solubility index (WSI), textural properties, thermal properties and pasting viscosity performed on the materials post extrusion. The microstructure was examined with scanning electron microscope (SEM) and epifluorescence microscopy to observe the cellular structures and components' interactions. These measured characteristics are important in predicting consumer's acceptability of extruded products.

## 6.2 Preliminary thermomechanical extrusions

The preliminary thermomechanical extrusions of white Agatu pigeon pea were studied using dry powder from milled pigeon pea with more than 60% of the particle sizes ranging between 125 - 355μm (section 3.2.3.1). Moisture was added to the barrel to obtain the low (12% and14%) and high (22%) moisture feed samples. These feed moisture levels are inclusive of the 11% moisture content of the dry milled powder. In addition to the different moisture levels, different extrusion conditions, as shown in Table 3-4and barrel segmented temperatures Table 3-6were applied. The same twin screw extruder described in section 3.3.2 was used throughout the preliminary extrusion process. A total of thirteen samples of extrudates were produced and numbered E1-E13 in Table 6-1. Nine of the extrudates (E1-E9) were obtained from high moisture (22%) feed, while the remaining four extrudates (E10-E13) were developed from low moisture (12 and 14%) feed.

### 6.2.1 Preliminary extrusion of high moisture (22%) native pigeon pea (NPP)

In the extrusion of the high moisture (22%) native pigeon pea, within the range of the extrusion conditions (temperature, screw speed, moisture content and feed rate) as shown in Table 3-4, some stable extrudates were produced. In addition to poor quality (physical features) of some of these extrudates (Figure 6-2), the expansion properties (bulk density and sectional expansion index) in Figure 6-1 were not satisfying. For proper evaluation, the product attributes of the nine extrudate samples (E1-E9) from this preliminary extrusion were subjected to analysis of the variance (ANOVA). A significant difference ($p<0.05$) existed within the product attributes responses on expansion characteristics (Figure 6-1). At higher die temperatures of 140 °C to 150 °C and screw speed of 300-400 rpm, the expansion characteristics of the extrudates (E6, E8 and E9) as indicated by lower bulk densities and higher expansion indices were improved. Although a structure (extrudate) has been formed, the expansion is low, despite the relatively high temperature at the end of the extruder.

**Table 6-1:** Effect of high and low moisture preliminary extrusion on extrusion process responses (SME and torque) and physicochemical properties of extrudates.

| Extrudate (E) | Die temp (°C) | Screw Speed(RPM) | Feed rate (kg/h) | Feed moisture content (%) | SME (Wh/kg) | Torque(NM) | Cold water viscosity (cP) | Hot-paste peak viscosity (cP) | WAI | WSI | Deformation force (N) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **High moisture (22%) extrusion** | | | | | | | | | | | |
| 1 | 120 | 200 | 8 | 22 | 26 | 20 | $34\pm2.39^{ab}$ | $2917\pm64.69^{g}$ | $4.36\pm0.18^{a}$ | $17.86\pm3.38^{ab}$ | NA |
| 2 | 120 | 300 | 8 | 22 | 35 | 21 | $27\pm6.01^{ab}$ | $2828\pm64.69^{g}$ | $4.55\pm0.35^{a}$ | $17.54\pm3.86^{ab}$ | NA |
| 3 | 120 | 400 | 8 | 22 | 44 | 18 | $26\pm2.08^{a}$ | $3522\pm66.25^{g}$ | $4.87\pm0.07^{abcd}$ | $20.89\pm1.81^{b}$ | NA |
| 4 | 140 | 200 | 8 | 22 | 28 | 16 | $39\pm16.46^{abc}$ | $437\pm21.00^{f}$ | $5.38\pm0.16^{de}$ | $18.27\pm0.14^{ab}$ | $70\pm24.59^{bc}$ |
| 5 | 140 | 300 | 8 | 22 | 35 | 16 | $59\pm4.04^{de}$ | $376\pm20.55^{ef}$ | $5.23\pm0.39^{bcde}$ | $17.59\pm0.05^{ab}$ | $76\pm13.51^{c}$ |
| 6 | 140 | 400 | 8 | 22 | 45 | 17 | $78\pm1.15^{ef}$ | $366\pm33.82^{ef}$ | $5.57\pm0.45^{de}$ | $17.67\pm0.62^{ab}$ | $59\pm18.99^{bc}$ |
| 7 | 150 | 200 | 8 | 22 | 25 | 19 | $46\pm4.93^{bcd}$ | $327\pm39.55^{de}$ | $5.34\pm0.31^{de}$ | $17.24\pm1.91^{ab}$ | $49\pm20.61^{b}$ |
| 8 | 150 | 300 | 8 | 22 | 31 | 30 | $57\pm3.05b^{cd}$ | $323\pm66.25^{de}$ | $5.48\pm0.19^{de}$ | $17.53\pm0.17^{ab}$ | $61\pm13.47^{bc}$ |
| 9 | 150 | 400 | 8 | 22 | 42 | 29 | $87\pm2.30^{e}$ | $304\pm14.57^{cde}$ | $5.78\pm0.08^{e}$ | $18.22\pm1.43^{ab}$ | $53\pm20.34^{bc}$ |
| **Low moisture (12% and 14%) extrusion** | | | | | | | | | | | |
| 10 | 150 | 200 | 8 | 14 | 39 | 24 | $36\pm1.00^{ab}$ | $238\pm33.06^{bcd}$ | $4.57\pm0.85^{abc}$ | $20.22\pm3.94^{ab}$ | $64\pm13.86^{bc}$ |
| 11 | 150 | 300 | 8 | 14 | 47 | 22 | $45\pm1.15^{abcd}$ | $216\pm13.31^{bc}$ | $4.44\pm0.08^{a}$ | $19.95\pm0.23^{ab}$ | $57\pm13.38^{bc}$ |
| 12 | 150 | 400 | 8 | 14 | 57 | 24 | $57\pm1.73^{cd}$ | $209\pm6.55^{b}$ | $4.82\pm0.32^{abcd}$ | $22.55\pm1.05^{b}$ | $61\pm11.74^{bc}$ |
| 13 | 150 | 400 | 8 | 12 | 63 | 24 | $115\pm13.52^{g}$ | $91\pm6.65^{a}$ | $5.08\pm0.06^{abcde}$ | $33.16\pm1.49^{c}$ | $13.53\pm6.47^{a}$ |
| NPP | NA | NA | NA | 11 | NA | NA | $36\pm3.21^{ab}$ | $3097\pm90.73^{g}$ | $4.87\pm0.07^{abcd}$ | $15.57\pm0.96^{a}$ | NA |

All data are mean value ± standard deviation of the triplicate analysis except deformation force which is means of 10 replicates. Mean with different letter in the same column differ significantly ($P<0.05$) with Tukey *post hoc* test.NA: Not applicable, NPP: Native pigeon pea powdered material.

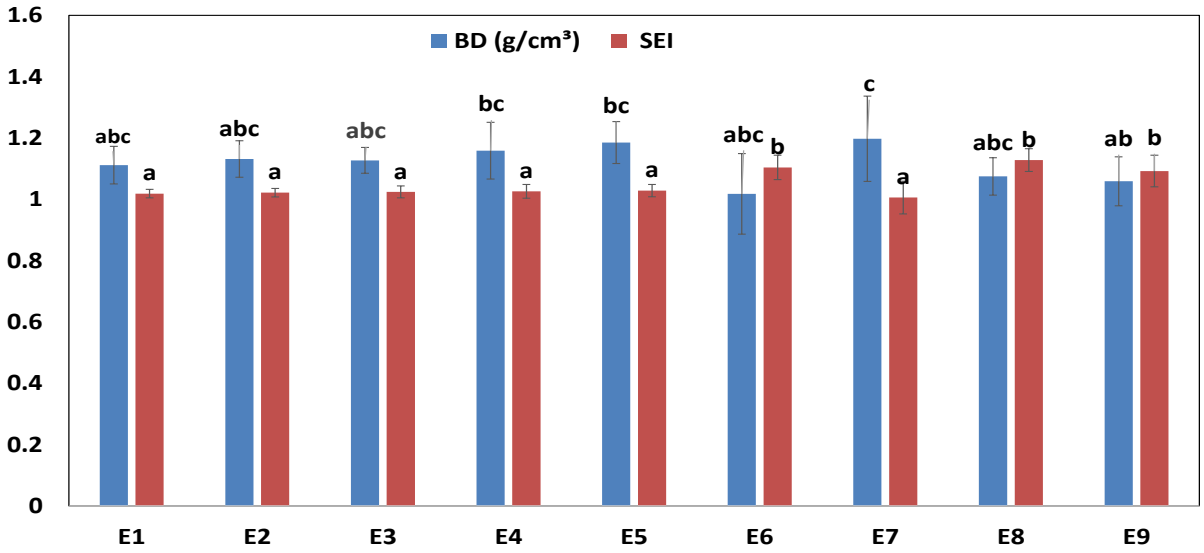



**Figure 6-1:** The expansion characteristics of extrudates from high moisture native pigeon pea feed.

SEI: Sectional expansion index, BD: Bulk density. The columns are the mean value of ten replicates. The error bar represents the standard deviation while the column with different letters are significantly different $p<0.05$ with Tukey *post hoc* test. For sample codes see Table 3.4.

**Key Result**

Temperatures up to 140 °C and screw speeds up to 400 rpm were considered the better conditions used in effecting some expansion of the products when a constant feed rate of 8 kg/h and high feed moisture content (22%) were used in the extrusion process.

Surprisingly, it was found that at high moisture extrusion, none of the selected extrusion conditions could increase the sectional expansion index of the Pigeon pea by about 5–fold. Hence, this would then have made it comparable to cereals (corn grits) that expanded to this level at the same extrusion conditions (unpublished Laboratory result from an initial pilot extrusion of pigeon pea with corn grits). The low expansion suggested that starch conversion was restricted during the extrusion. Legume starch conversion does seem to differ from cereal. Contrasting reports on expansion and starch conversion of composite feeds from pigeon pea and

other legume flours exists. Among an investigated report (Silva et al., 2014), higher degree of starch conversion and expansion were obtained when lower proportion (15%) of dehulled carioca bean flour was extruded with higher blend of corn grits at lower (13%) feed moisture content. Also, the crispness of extruded product from dehulled pigeon pea were improved at lower blends (5% and 10%) of pigeon pea flour with cassava and fermented maize flour respectively (Rampersad et al., 2003, Fasoyiro et al., 2017). A higher sectional expansion index of 5.5 was reported in a blend of 15.7% pigeon pea with millet at high moisture content (19%).

The hardness (texture characteristics) of the extrudates, represented as the maximum peak deformation force, was determined by textural analyses (section 3.4.4). The maximum peak deformation force in Table 6-1was measured as the force required to puncture about 60% of the extrudate's cross sectional diameter. This force is also related to the force required by the teeth in the mastication (chew/bite/fracture) of the product. The measurement of the high forces required to penetrate the extrudates reflects the poor expansion. Values for the samples created at the lowest temperature (120 °C) are not given, as these samples would not fracture within the operational load of the texture analyser's load cell (30 kg load cell). For the rest of the samples created they showed similar break strength (maximum deformation force).


**Figure 6-2**: Extrudate (E9) from the preliminary extrusion at high moisture content (22%), die temperature (150 °C) and screw speed (400rpm). The appearance of the sample E9 as a cohesive string of material and the small sectional diameter confirmed the poor expansion characteristics**.**

Although, extrudates E9 in Figure 6-2were produced from high moisture (22%) feed but the expansion characteristics were expected to improve even at higher die temperature (150 °C) and screw speed. The cohesive string feature of this sample (half product) and exterior surface that is filled with entrapped creamy particles confirmed the poor product quality of the sample. The entrapped particles were probably un-melted feed materials that were embedded into the continuous melt matrix during the extrusion. This non-molten feed particles, are likely to be predominantly starch and may be the indication of why the non-expansion of the extrudate occurred. Evidence of the non-expansion was also shown by the extrudate diameters nearly corresponding to the die diameter and this is shown in the low values of SEI.

As the screw speed (given in Table 6-1increased, the calculated specific mechanical energy (SME) in the system increased, but the torque required

was not noticeably higher. There is much doubt also whether the SME values calculated are numerically correct. The equipment manufacturers have been contacted on several occasions, but the issue of the high calculated SME's has not been resolved. Hence the percentage values for these values (SME and torque) are probably a better indicator of performance rather than actual values. It is possible that at a high moisture extrusion, free non-hydrating moisture enhanced the flow of the non-molten material so that little energy (SME) was utilised in the development of the extrudate.

For direct expansion to occur it is necessary to convert the starch within the continuous matrix (section 2.6.1). To investigate the state of the starch, the RVA pasting viscosities (cold water and hot paste peak viscosities) were determined. The results of the extruded material were compared with the native pigeon pea (NPP) that had not undergone thermomechanical breakdown. During the determination of the RVA pasting, the extrudates were dried and then milled in a similar manner as the uncooked pigeon pea. It was noted that the extrudates, particularly those formed at 120 °C had 18% moisture content before oven drying and this makes them very dense and difficult to mill. The milled samples which passes through 355 µm sieve size were then added to water and their viscosity estimated using a rapid visco analyser (section 3.7.2). The RVA curves of some extrudates from the high moisture feed material are shown in Figure 6-3 to illustrate the level of starch conversion during the thermomechanical extrusion.

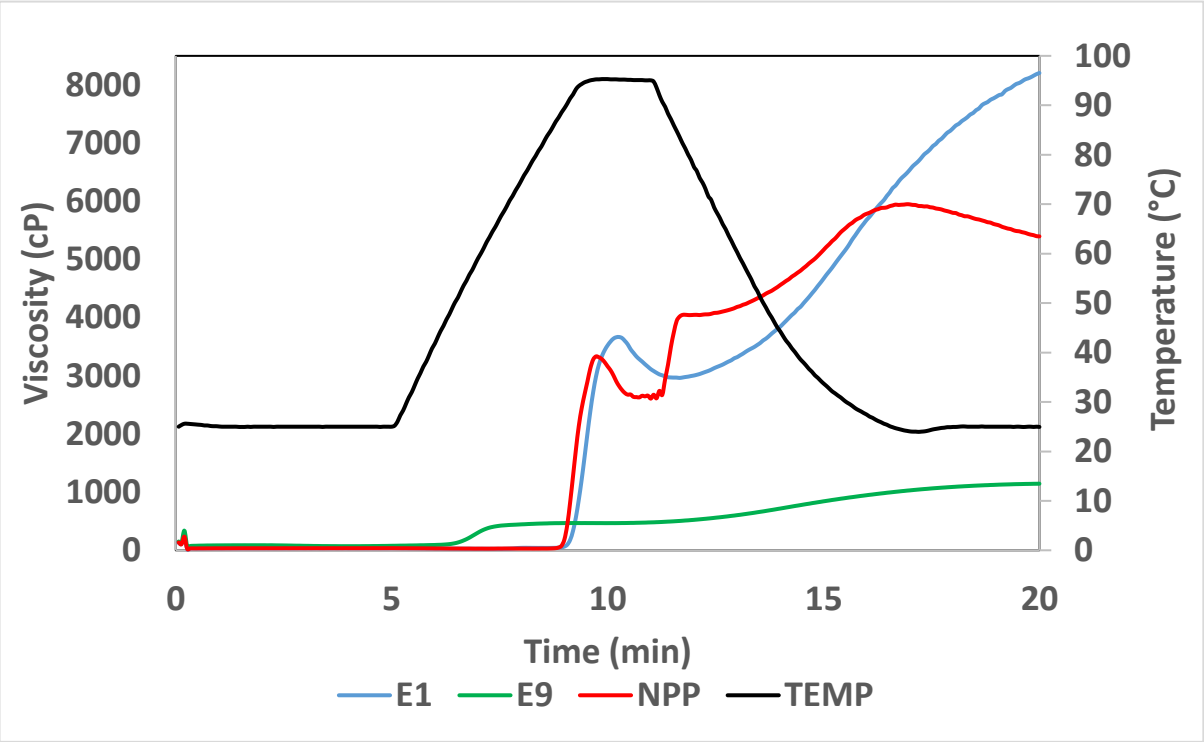



**Figure 6-3*:*** RVA curve of native pigeon pea (NPP) and extrudates (E1 and E9) from high moisture content (22%) feed material and constant feed rate of 8 kg/h.

E1: minimum processed at 120 °C die temperature, 200 RPM screw speed and E9: maximum processed at 150 °C die temperature, 400 RPM screw speed.

In Figure 6-3, E9 showed some low temperature swelling (cold water swelling), which occurred after 5 minutes of pasting at the lowest pasting temperature of 25 °C. This sample never showed much viscosity as some of the starch has been converted. The other extrudate (E1) required higher temperatures to swell and produce a measurable viscosity. The major viscosity peaks (hot paste viscosity) occurred when the particles were heated to temperatures more than 90 °C for the native (NPP) and the 120 °C (E1) extrudates. This would normally be considered evidence of low starch conversion, but as the extrudates were so difficult to mill, the powders created may not be representative of the internal structures of the extrudates.

The RVA pasting profiles did alter when the extrusion temperature increased from 120 °C to 150 °C (Figure 6-3 and

Figure 6-4). For samples extruded at above 120 °C (E9), the hot paste viscosities are much lower than observed in the native and 120 °C samples. Although these samples extruded at above 120 °C (E9) had been subjected to comparable mechanical energies, but starch conversion seemed to be favoured by greater thermal inputs. Despite the behaviour in water of extrudate particulates being different when observed using the RVA for the higher heat treatment samples (E9), their expansion was still low and the behaviours of all samples when in cold water in terms of water solubility and swelling were similar (Table 6-4). At this level of higher heating (150 °C) and mechanical force used in production of sample E9, melting of the starches could be expected, but changes seem limited. To form a continuous melted matrix, which can be expanded by the rapid change from super-heated water into steam at the die, the starch needs to lose its native structures.

Low frictional forces are one of the difficulties that could occur by extrusion at high moisture feeds as the slippage of the viscous mixtures reduces the material interactions within the barrel. This may have been expected for the pigeon pea due to its difficult hydration characteristics. If water is not entering the particulates, then it would form a coating around the particles allowing slippage and lubrication of the material as it passes through the extruder. Although temperatures are high within the extruder, the moisture contents within the pigeon pea powders may be very low and therefore the typical melting of the starch granules may not occur. It is suggested that at 10% moisture and 180 °C, the melting of many starches during

thermomechanical extrusion would be in excess (2.6.3)(Lai and Kokini, 1991).

In thermomechanical extrusion, most of the starch conversion is thought to come from the mechanical forces and as these increase, the temperatures at the tips of the flights of the extruder screws can be 40 °C higher than the mean barrel temperature (Della Valle et al., 1987). Therefore, if the particles do not hydrate during high moisture extrusion, two factors may be occurring limiting the starch conversion. First, low water content in the powders (due to delay in water absorption) reducing the probability of thermal conversion and secondly, lubrication limiting mechanical disruption of the powders and the starch granules. Since, extrusion at 22% moisture would be considered too high for most materials expected to undergo direct expansion, the decision was that in the next piece of experimental work the moisture content would be reduced to 14 and 12%.

### 6.2.2 Preliminary extrusion of low moisture (12 & 14%) NPP feed

From the first preliminary extrusion cooking with high moisture content feed (section 6.2.1), it was established that native pigeon pea powder could be extruded into direct expanded product, but expansion was poor. To optimise the extrusion cooking and obtain more stable product, a second extrusion trial was carried out at low moisture contents of 14% and 12% using a die temperature of 150 °C and feed rate of 8 kg/h. The data from this trial are shown in Table 6-1 and from

Figure 6-4 to Figure 6-7.

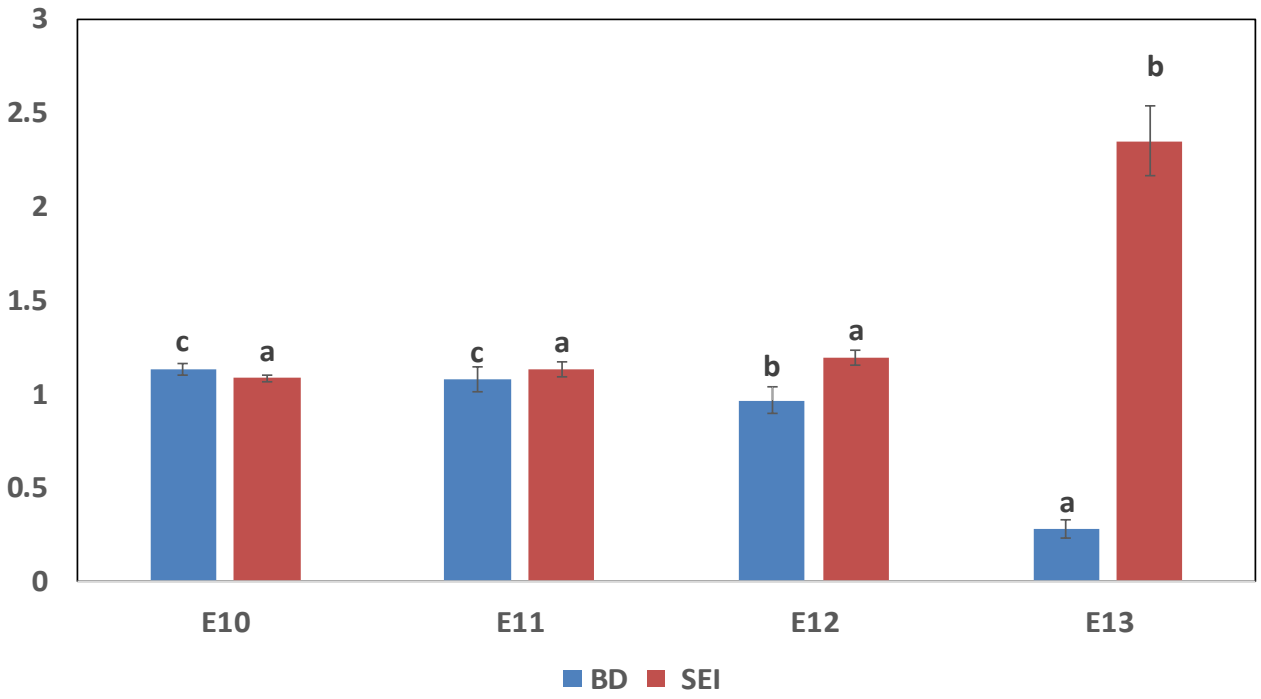



**Figure 6-4** The expansion characteristics of extrudates from preliminary extrusion of low moisture native pigeon pea feed.

See Table 6 1 for extrudate abbreviation and extrusion conditions). The columns are mean value of ten replicates. The error bar represents the standard deviation while the column with different letters are significantly different. When compared to E12 at the same extrusion condition, further reduction of moisture enhanced expansion of E13 (SEI: Sectional expansion index, BD: Bulk density).

AS in E13, the further reduction in moisture content (12%) with increase in temperature (150 °C) and screw speed (400 rpm) did seem to reduce the slippage effect as evidenced by an increase in torque and more than a doubling of the SME (Table 6-1) when compared to the equivalent sample at 22% moisture. With this increase in SME, it did seem possible to produce

extrudate with higher expansion index and low bulk density as shown by E13 in

Figure 6-4. Although the expansion index (100% increase in diameter compared to die diameter) was still lower than what was obtained in a similar extrusion condition with 30% pigeon pea + 70% corn grit which expanded 5-fold relative to the die diameter (unpublished Lab result from an initial pilot extrusion of pigeon pea with corn grits). Many authors (Ferreira et al., 2011, Robin et al., 2011) have observed low expansion index (5-fold increase in sectional diameter) in high protein–fibre direct expanded snacks when compared to cereal extrudate that shows more than a 10-fold increase in expansion. The low expansion in high fibre-protein


extrusion has been attributed to high fractions of the non–starch components, which reduces the starch fraction in the feed material and starch conversion in the extruder.

Figure 6-5:**Comparison of extrudates from low moisture (E13) and high moisture (E9) extrusion.**

Extrudate E9 formed as a string structure with low sectional diameter shows a white patchy exterior surface while E13 from low moisture extrusion appeared with rough jagged-saw (shark-skin) exterior surface in addition to high sectional diameter. This rough exterior surface of E13 may be due to instability of the expanding bubble wall which causes an early disruption of cells during the exit of the melt from the die pressure.

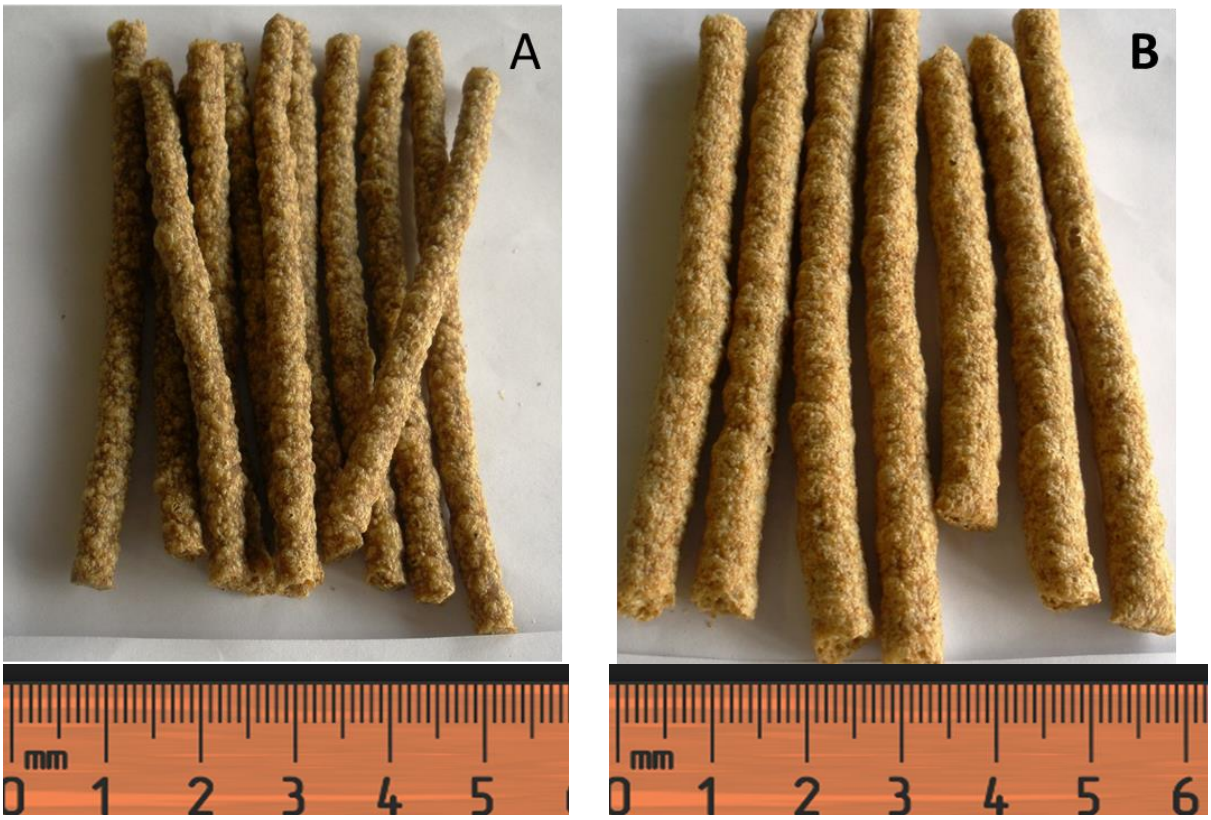



**Figure 6-6:**Comparison of two extrudates produced from the same die temperature, screw speed, feed rate but different moisture content.

(A) Sample E12 produced with 14% feed moisture content (B) sample E13 produced with 12% feed moisture content. Through an increase in SME from the further reduction of feed moisture to 12%, extrudate expansion was improved**.**

However, from the observation of the physical features of sample E13 in Figure 6-6, a more stable structure has resulted compared to all the preliminary extrusion samples from high moisture extrusion. Sample (E12) external surface seems compacted compared to E13 with higher SEI (2.35) as shown in

Figure 6-4. Since this product (E13) from the lowest feed moisture (12%) recorded the highest SEI, it became clear that less water was required for extrusion of pigeon pea.

Following the methodology in section 3.4.4, the texture of the extrudates was tested. The maximum peak deformation force in Figure 6-12 varied as the feed moisture content reduces. Sample E13 had the lowest maximum peak deformation force compared with the rest of the extrudates in this second preliminary extrusion. Generally, it has been reported that the cellular structure of an extrudate influences the maximum peak deformation force necessary to deform the extrudate. Both the size, number and area covered by the air cells determine how large or small the force deformation peak would be (Robin et al., 2010, Chanvrier et al., 2013). Therefore, the lowest deformation peak force shown for the sample created using the lowest feed moisture, highest screw speed and temperature is a good indication that less force is required during chewing/biting of this extrudate. An important attribute accepted by consumers of edible solid foam.

Lowering the feed moisture content seems not to change the water absorption index (WAI) compared to the native pigeon pea (NPP). However, water solubility index (WSI) was mostly increased by low moisture

extrusion. It could be expected that increase in WSI arises from increase in the leaching of starch soluble components from the native amorphous components and the melted crystalline components following starch degradation during extrusion cooking. Despite the water absorbance of E12 and E13 not showing an effect compared with high moisture extrusion, there is an increase in cold water viscosity as demonstrated from the RVA curve in Figure 6-7 of sample E13 produced from the least feed moisture (12%) and high screw speed sample.

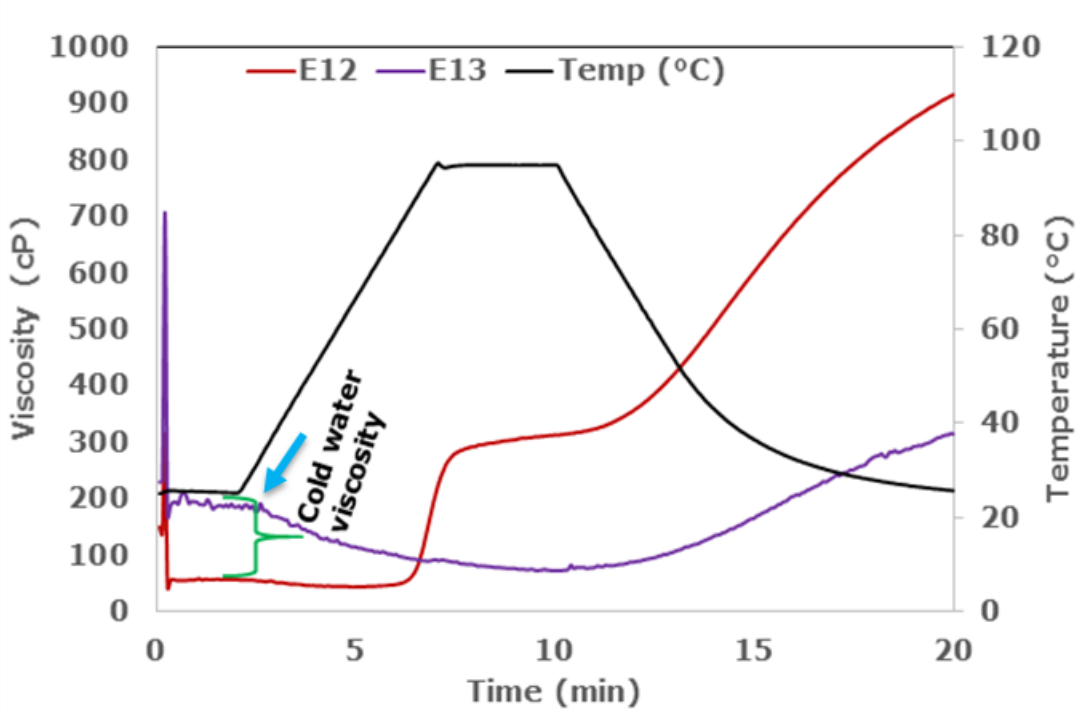



**Figure 6-7*:*** RVA curve from preliminary extrusion of extrudates from low moisture content 14% (E12) and 12% (E13) feed, processed at 150 °C die temperature and 400 rpm screw speed.

The RVA profile shows increase in cold water viscosity of E13 due to melting of starch crystalline structure during the low moisture feed extrusion cooking.

The high cold water viscosity of E13 in the RVA curve (Figure 6-7) seem to indicate that there could be loss of pigeon pea starch crystallinity during

low moisture extrusion and thus allowing the starch to hydrate and swell without heat during pasting within the RVA. The starch structure does seem to be disrupted during the extrusion process when compared to the starch behaviour as described in the seed softening treatment in previous chapter 5 where the starch crystalline structure resisted melting during quiescent hydration and heating treatments. Therefore, extrusion cooking has the best ability to alter the thermo-stable pigeon pea seed to form a ready to eat snack product.

### 6.2.3 Conclusion on preliminary extrusion

Although, during the preliminary extrusion of high moisture feed, all samples had been subjected to comparable mechanical energies but starch conversion, as shown by RVA curve, was favoured by greater thermal inputs (increase in temperature to 150 °C). Further results showed that the product (Figure 6-6B) formed with the lowest feed moisture content (12%), at higher temperature (150 °C) and screw speed (400 rpm) had the highest sectional expansion index (SEI) and cold water peak viscosity. This could be an indication that less water is favourable for loss of the pigeon pea crystalline structure during thermomechanical extrusion. Possibly, due to the short residence time in the extruder and the delay in the hydration of pigeon pea, the addition of water during extrusion may result in the lubrication of the feed instead of plasticisation. Hence the lubrication will reduce the frictional force required for the melting of starch crystalline structure and homogenisation of melt flow.

At the stage of the preliminary extrusion trials, it was still difficult to detect the contributions of the non-starch components in the extrudates, hence, this would require further analysis. Though the results from preliminary extrusion trials, at low moisture content, indicated that ready to eat expanded extrudates from pigeon pea (as seen in Figure 6-6) were possible with features of poor product quality in some extrudates. This preliminary

extrusion suggested that further work aimed at improving the hydration of feed was necessary for the creation of an acceptable expanded product.

## 6.3 Thermomechanical extrusion with treated feed

The preliminary extrusions described in section 6.2 resulted in extrudate with less expansion compared to cereal at the same conditions. This indicated the challenges of using the thermomechanical energy and heat for starch conversion in the presence of the rest of the materials and structures occurring in pigeon pea. A repeat of the preliminary extrusion was carried out with some modifications aimed at increasing the mechanical work through increasing the feed rate from 8 kg/h to 14 kg /h. The increase in feed rate would ensure better filling of the extruder barrel as maximum feed rate from the manufacturer was suggested to be 20 kg/h. It is also expected to reduce slip and pressure behind the die. The TSE 24 MC twin screw extruder previously described in section 3.4.2 was used throughout these studies.

Two extrusion feeds were prepared from the milled native pigeon pea. They included a low moisture feed, which was the same as initial low moisture (11%) milled native pigeon pea seed (extruded as control) and a high moisture (22%) feed, obtained by addition of water to the 11% milled native pigeon pea in the extruder barrel. During the extrusion, a constant die temperature of 150 °C and screw speed of 400 rpm were maintained.
As the ground native pigeon pea powder seemed difficult to hydrate within the time frame of the extrusion (the short residence time of 22 secs), a pre-hydration step was considered. As described in Chapter 5 this was shown to be beneficial for hydration. The treatment (HTT) chosen was the hydration of the seed at 90 °C for 5 min in a jacketed vessel with continuous stirring. Following the hydration treatment, the hydrated seeds had increased in moisture to 22% and then were wet milled (HTTHME) after cooling in the cooled jacketed vessel and used directly as the extruder feed.

To obtain the low moisture feed from HTT, the hydrated 22% moisture seeds were then dried at 50 °C (section3.2.3.4) and dried milled. The resulting powder (HTTE) had a moisture content of 11% dwb and these was used directly as dry feed. No further water was added in the barrel during extrusion.

In the last batch of the feed preparation, the seeds had undergone the HTT to hydrate and soften the seed components, but to enhance cellular disruption, the hydration occurred in 0.5% sodium bicarbonate (alkaline) solution (section 3.3.1) with an increase in the seed moisture content to 22%. Like the previous HTT, some portion of the hydrated(22% moisture content) seed in the sodium bicarbonate (alkaline) solution were wet milled (BBHME)while the other (BBE) was dried to 11% moisture content and then milled.

Some product characteristics such as water absorption index (WAI), water solubility index (WSI), maximum peak deformation force, cold water viscosity and SME were evaluated on the formed extrudates as shown in Table **6-4**. For convenience Table **6-2** is also given as a fold out page in Appendix A so that the details represented by the codes can be viewed alongside the data.

**Table 6-2:**Sample codes for the thermomechanical extrusion of non-treated and treated pigeon pea powders

| Sample code | Non-treated native pigeon pea(NPP) | Hydrothermal treatment (HTT) | Alkaline treatment with bicarbonate (BB) | Wet (W) or Dry (D) milling | Moisture content of the feed (% DWB) | Moisture (%) addition in the barrel |
|---|---|---|---|---|---|---|
| NPPHME | Yes | No | No | D | 11 | Yes |
| NPPE | Yes | No | No | D | 11 | No |
| HTTHME | No | Yes | No | W | 22 | No |
| HTT | No | Yes | No | D | 11 | No |
| BBHME | No | Yes | Yes | W | 22 | No |
| BBE | No | Yes | Yes | D | 11 | No |

### 6.3.1 Formation of extrudates from treated feeds

The thermomechanical extrusion of low moisture treated feed resulted in the formation of more stable extrudates compared with preliminary extrusion (section 6.2). The improved stability of the products accounted for the differences in their physical appearance. The camera pictures of the extrudates (Figure 6-8) confirmed the structural changes which may be because of the difference in the feed material's hydration properties following the treatment.

The extrusion responses in Table 6-3 shows that at higher moisture content and feed rate, the torque required to rotate the screw and the feed decreases and consequently there is a lowering of the SME (Akdogan, 1996). In contrast, at low moisture extrusion of both treated and non-treated materials, the increase in torque increased the SME. An indication that as the workload increases on the screw, the torque also increases.

**Table 6-3*:*** Extrusion responses during the extrusion of treated and non-treated feeds. Sample description can be found in Table 6-2

| Sample | Torque (Nm) | Pressure (Bar) | Temp (°C) of last barrel zone (zone 10) | Melt temp | SME (Wh/kg) |
|---|---|---|---|---|---|
| NPPHME | 19 | 34 | 120 | 158 | 28 |
| NPPE | 53 | 21 | 121 | 70 | 79 |
| HTTHME | 23 | 8 | 120 | 63 | 34 |
| HTTE | 52 | 21 | 123 | 66 | 77 |
| BBHME | 25 | 23 | 120 | 76 | 37 |
| BBE | 68 | 23 | 132 | 67 | 101 |

The extrusion of the low moisture alkaline treated feed showed the highest torque in contrast to high moisture preliminary extrusion (Table 6-1) at a lower constant feed rate of 8 kg/h, higher temperature (150 °C) and screw speed (400 rpm). Due to the inconsistency of torque at the two high moisture extrusions (preliminary and present studies), it seems that the torque depends on modifications of the viscoelasticity of the extruding material as it changes from solid elastic material to rubbery-liquid.


**Figure 6-8:** A-C: extrudates from high moisture (22%) feed (A) non-treated (B) hydrothermally and (C) alkaline treated feed, D-F extrudates from low moisture (11%) feed (D) non-treated, (E) hydrothermally and (F) alkaline treated

### 6.3.2 Effect of feed treatment on extrudate expansion properties

As shown in Figure 6-9, the expansion properties of extrudate from treated feed were improved compared to extrudates from non-treated feed. The expansion index significantly (P<0.05) increased from 1.09 in NNPHME to 3.42 in BBE while the bulk density decreases most in BBE. In addition to the image of NPPHME in Figure 6-8 that indicates the extrudate being similar to the size of the extruder die, the low SEI and high BD are evidence of limited starch conversion. Usually such an extrudate is termed a half product and it requires a second process for the starch crystalline structure to be fully melted and sufficient starch conversion to occur to achieve product expansion.

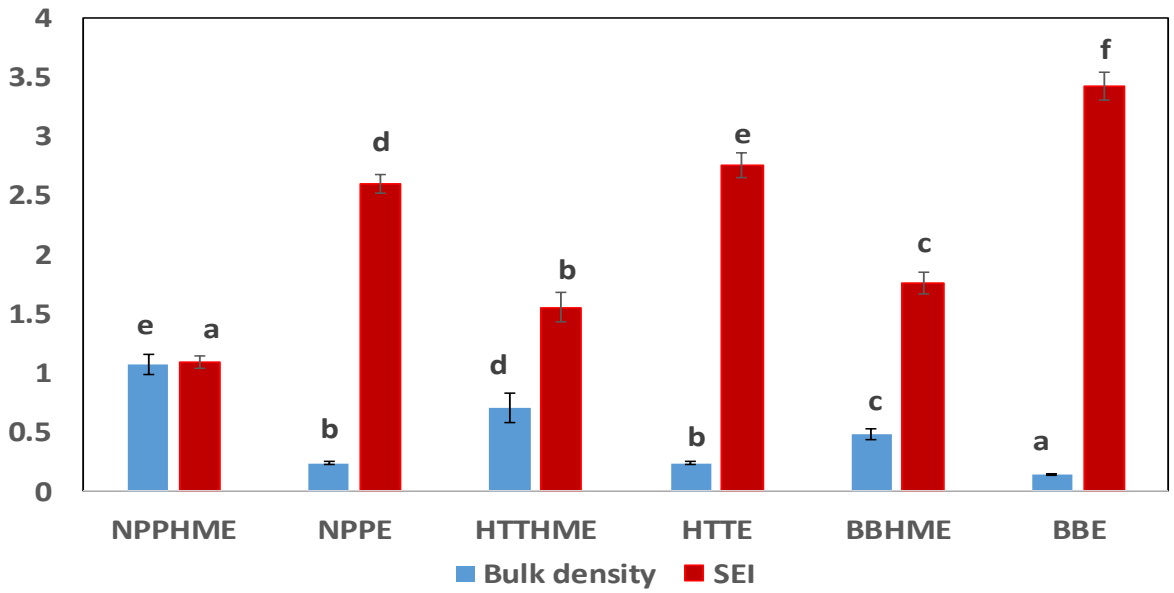



**Figure 6-9:** Expansion properties of extrudate from treated feed (SEI: Sectional expansion index, BD: Bulk density, MC: Moisture content). The columns are mean value of ten replicates. The error bar represents the standard deviation while the column with different letters are significantly different (P<0.05).
NPPHME: extrudate from high moisture (22% MC) native pigeon pea, NPPE: extrudate from (11% MC) native pigeon pea, HTTHME: extrudate from wet (22% MC) milled HTT treated pigeon pea, HTTE: extrudate from dry (11% MC) milled hydrothermally treated pig`1eon pea, BBHME: extrudate from wet (22%MC) milled alkaline treated pigeon pea, BBE: extrudate from dry (11% MC) milled alkaline treated pigeon pea.

The Pigeon pea extrudate can be considered a complex extrudate due to the basic components which consists of starch (45%), protein (20%) and fibre (26%). It is expected that the non-starch components, when not-treated, would act as a diluent and will influence the expansion (Moraru and Kokini, 2003). Although the hydrothermal and the drying treatment that follows made no difference to the expansion of HTTE compared to NPPE. However, considerable differences were observed in SEI and BD after alkaline treatment in extrudate BBE compared to preliminary extrusion of 14% low moisture feed (section 6.2.2) at higher temperature and screw speed. Since, the expansion properties of starch based extrudate is related to the extent of starch conversion, extrusion of the alkaline (sodium bicarbonate) treated feed material at low moisture content seems to increase the starch conversion which favours increase in SEI and decrease in BD. Contrasting effects of the expansion of extrudates following sodium bicarbonate treatment of extrusion feeds has been reported. For example, it was stated that addition of sodium bicarbonate to bean flour feed was effective in increasing expansion ratio to two-fold. The mechanism of expansion by sodium bicarbonate in the bean flour extrudate was attributed to the numerous air cells created by the evolution of $CO_2$ from sodium bicarbonate at temperature higher than 100 °C (Berrios et al., 2004). While the effect of sodium bicarbonate in wheat flour extrusion was observed to have uneven influence on bulk expansion properties(Robin et al., 2010).

Two major phenomena are known to control expansion at die exits. They are expansion through vapour flash-off and by die swell. The die swell, which is more prevalent in synthetic extrusion, occurs due to elastic recovery of deformation on the extruding melt. In food, it is a characteristic of melts with low gas bubble units and those occurring at lower temperature (approximately 100 °C)that prevents the flash-off of vapour (Della Valle et al., 1997, Moraru and Kokini, 2003). If a product swells at the die, to hold the expanded form, the product must regain a stable state whilst expanded. For biopolymers, this normally means loss of water acting

as the plasticiser, and lowering of temperature so the product changes from a rubbery material into the stable glassy state.

### 6.3.3 The functional properties of extrudate from treated feed

The hydration behaviour of the extrudate at room temperature (20 °C) and with little shear, was determined as an indication of the functional properties of the extrudate. The method for the water absorption index (WAI) is described in section 3.4.3 and the results are given in Table 6-4. Among the treated and non-treated extrudates, no definite trend was observed in the cold water absorption index. The WAI did not correlate with the expansion index. Generally, as starch loses its crystalline structure it is able to associate with water and the WAI increases. The loss of native crystalline starch structure therefore would show high WAI whereas low transformation of the starch granules or highly degraded starch macromolecules will exhibit low WAI. All the water absorption indices were low for the extudates produced at low moisture content, but those for NPPHME and HTTHME produced at higher moisture content were significantly higher ($p<0.05$). For the assessment of the WAI, it is necessary to grind the extrudates to powders and it may be that these poorly expanded extrudates showed differences in their powdered forms and this could have influenced the apparent water uptake.

The more, the extrusion cooking solubilises and degrades the melt components (starch, protein and fibre), the more the extrudate's WSI would be expected to increase. The WSI results in Table **6-4** followed the trend BBE>BBHME>HTTE>NPPE>HTTHME>NPPHME>NPP. The high WSI for BBE (extrudate from low moisture alkaline treated feed material) compared with the low value for NPP (native pigeon pea) and this may confirm that some of the starch was degraded during the extrusion cooking.

**Table 6-4:** Effect of extrusion cooking on characteristics of extrudate from treated and non-treated material.

| Sample | Deformation force (N) | WAI | WSI | Cold water viscosity (cP) | Peak viscosity (cP) |
|---|---|---|---|---|---|
| NPP | NA | 3.34±0.23$^{ab}$ | 17.96±0.51$^{a}$ | 36.66±2.49$^{a}$ | 3162.33±17.13$^{e}$ |
| NPPHME | 28.70±12.06$^{bc}$ | 5.78±0.08$^{d}$ | 18.22±1.43$^{a}$ | 75.66±1.24$^{a}$ | 467.66±14.57$^{b}$ |
| NPPE | 2.1±0.49$^{a}$ | 4.19±0.13$^{c}$ | 30.86±1.54$^{c}$ | 441.33±13.02$^{b}$ | 168.33±8.5$^{a}$ |
| HTTHME | 35.57±10.69$^{c}$ | 6.23±0.43$^{d}$ | 23.43±1.48$^{b}$ | 3195±41.73$^{d}$ | 1069.60±14.04$^{c}$ |
| HTTE | 18.88±3.30$^{b}$ | 3.27±0.23$^{a}$ | 32.37±0.74$^{c}$ | 811.33±42.08$^{c}$ | 494.66±34.64$^{b}$ |
| BBHME | 50.18±8.78$^{d}$ | 4.19±0.27$^{c}$ | 40.77±1.30$^{d}$ | 899.66±10.47$^{c}$ | 1661.66±13.59$^{d}$ |
| BBE | 6.5±1.39$^{a}$ | 4.01±0.09$^{bc}$ | 42.85±0.74$^{d}$ | 849.33±12.81$^{c}$ | 357.66±6.42$^{ab}$ |

Data are mean value of the triplicate analysis (except in deformation force where 10 replicates were used) ± standard deviation. Means with different letter in the same column differ significantly ($p<0.05$).

NA: not applicable. MC: Moisture content. NPPHME: extrudate from high moisture (22% MC) native pigeon pea, NPPE: extrudate from (11% MC) native pigeon pea, HTTHME: extrudate from wet (22% MC) milled HTT treated pigeon pea, HTTE: extrudate from dry (11% MC) milled hydrothermally treated pigeon pea, BBHME: extrudate from wet (22% MC) milled alkaline treated pigeon pea, BBE: extrudate from dry (11% MC) milled alkaline treated pigeon pea.

### 6.3.4 The thermal properties of the extrudates produced from the treated and non-treated feed material

The thermal properties of milled extrudate powder were determined using DSC (DSC823, Mettler Toledo, Switzerland). The details of the methods for these determinations are available in (section 3.7.3). The thermal properties of the extrudate indicates the enthalpy (energy required) and temperature necessary to gelatinise the remaining native starch in the extrudate after extrusion. It is a measure of the overall crystallinity of the starch granule (Garcia and Lajolo, 1994). The evaluation of starch thermal properties using the DSC analytical technique used in this work was dependent on hydration of the sample before the analysis. Results in Table **6-5** shows that thermal properties of the Pigeon pea were altered after extrusion.

No gelatinisation enthalpy (100% reduction of native starch enthalpy) was measured for the samples that had been pre-heated and formed the extruded samples of BBE and BBHME. The pre-treatment itself did not cause loss of starch order and in fact higher gelatinisation enthalpy values existed in the feed materials of BBE and BBHME when compared to the native pigeon pea (NPP). However, the prior alkaline treatment of the feed must have assisted in the loss of the starch crystalline structure during the extrusion.

**Table 6-5:**Effect of extrusion cooking on DSC thermal properties.

| Sample | Gelatinisation enthalpy of feed material (J/g) | Gelatinisation enthalpy (J/g) of extrudate | % change (reduction) in extrudate gelatinisation enthalpy from native pigeon pea | % change (reduction) in extrudate gelatinisation enthalpy from feed material | Onset (°C) | Peak (°C) | Endset (°C) |
|---|---|---|---|---|---|---|---|
| NPP | 5.4 | NA | NA | NA | 80±0.56 | 88±0.12 | 105±1.76 |
| NPPHME | 5.4 | 3.78 | 30 | 30 | 79±0.33 | 85±0.08 | 97±0.31 |
| NPPE | 5.4 | 1.73 | 68 | 68 | 47±0.00 | 69±0.42 | 103±0.00 |
| HTTHME | 6.66 | 0.91 | 83 | 86 | 50±3.44 | 69±0.37 | 94±4.57 |
| HTT | 6.66 | 2.02 | 63 | 70 | 47±0.22 | 70±0.41 | 103±0.00 |
| BBHME | 6.17 | ND | 100 | 100 | ND | ND | ND |
| BBE | 6.17 | ND | 100 | 100 | ND | ND | ND |

Data are mean value of duplicate analysis, ± standard deviation. ND: Not detected, MC: Moisture content. NPPHME: extrudate from high moisture (22% MC) native pigeon pea, NPPE: extrudate from (11% MC) native pigeon pea, HTTHME: extrudate from wet (22% MC) milled HTT treated pigeon pea, HTTE: extrudate from dry (11% MC) milled hydrothermally treated pigeon pea, BBHME: extrudate from wet (22% MC) milled alkaline treated pigeon pea, BBE: extrudate from dry (11% MC) milled alkaline treated pigeon pea.

When compared with the native (NPP), the thermal properties of other extrudates show a reduction in gelatinisation enthalpy, although some order is retained. The highest gelatinisation enthalpy remaining after extrusion occurred in sample NPPHME where only 30% reduction in enthalpy occurred after the extrusion. This is an indication that the extrusion conditions were not sufficient to damage all the starch granules and some starch crystalline structures were not melted during the extrusion process. It would be expected that in extrusion not only would the native starch structure be altered, but even the macromolecular structure would be depolymerised. Similar to other legume starches, pigeon pea starch granules are characterised by the close dense packing of the crystallites, which provides a structural stability, and limits accessibility of water during gelatinisation. Also due to the higher proportion of long amylopectin chains, the crystallites are more resistant to mechanical share during processing (Singh et al., 2008b).

It is possible that all or a portion of some of the gelatinisation enthalpies noted for the extrudates was not from the native starches but due to retrogradation having occurred. The low onset temperatures (<55 °C) noted for several of the extrudates would be indicative of a retrograded sample.

### 6.3.5 The effect of feed treatment on extrudate pasting properties

To assess the levels of starch conversion during the extrusion cooking of treated and non-treated feed material, the extrudates were milled and the powder of particle size which passes through 355 µm sieve was dispersed in cold water and pasting viscosities measured under RVA time temperature-shear regimes3.7.2).The RVA curves shown in Figure 6-10 indicates if cold water viscosity developed within 5 min of pasting at 25 °C (the lowest temperature of the profile) and the hot paste peak viscosity was displayed during 10 min of pasting at 95 °C.

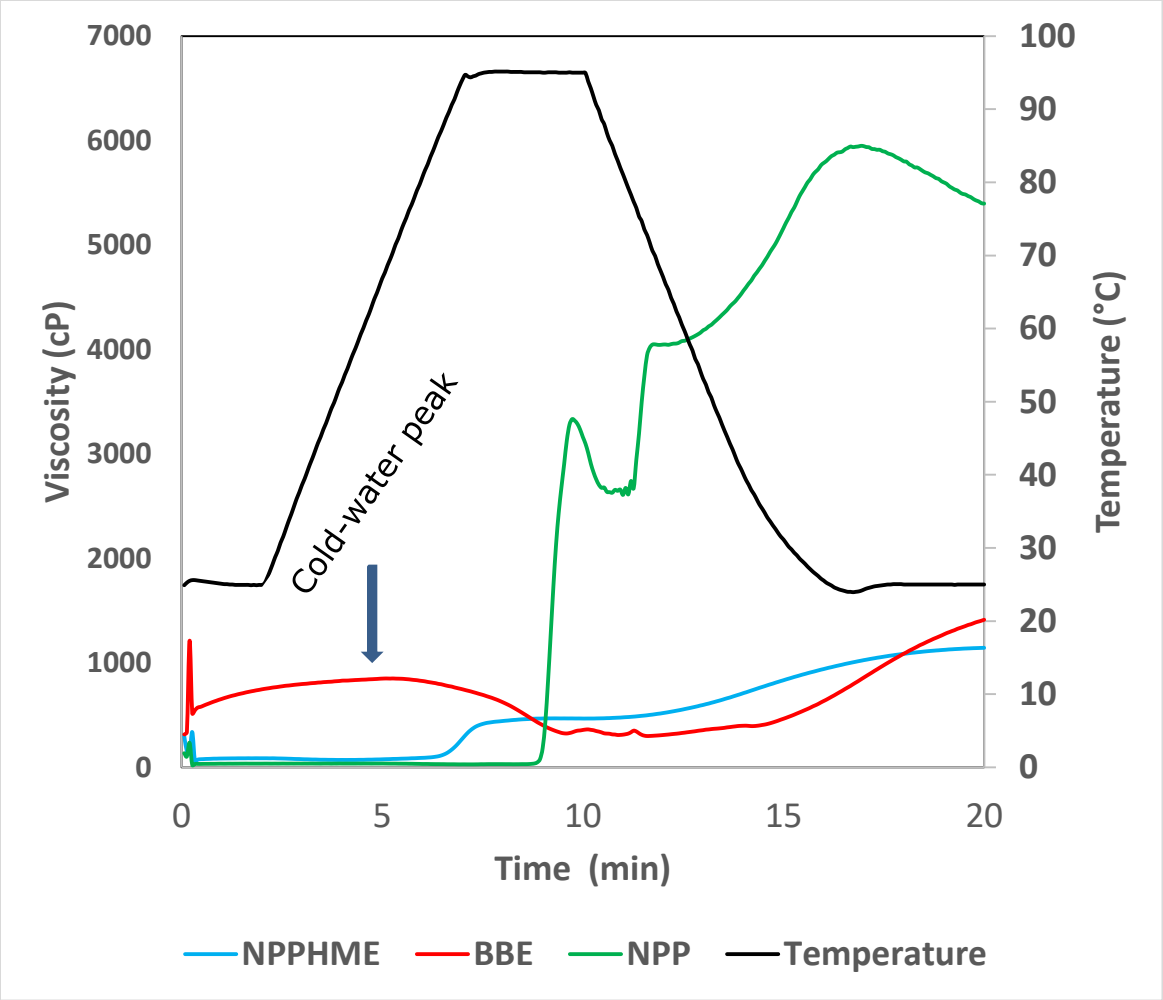



**Figure 6-10:**Pasting curve showing cold and hot water viscosities of native pigeon pea (NPP), and extrudates from high moisture non-treated native pigeon pea (NPPHME) and low moisture alkaline treated (BBE) feed.

BBE developed high cold peak following the high starch conversion from the extrusion of feed with high degree of cell wall degradation.

A cold water viscosity at 25 °C, 5 min and low hot peak viscosity were exhibited by extrudate BBE created using the low moisture (11%) treated feed. This type of profile is often an indication of starch without crystalline structure. NPP without any measurable cold water viscosity and exhibiting a high peak viscosity, following the gelatinisation of starch during pasting, is as expected for a product containing native starch. The absorption of water and swelling of granules after disruption of the native crystalline structure during pasting results in the formation of peak viscosity. For this legume product (NPPHME) the maximum swelling occurs during the pasting

phase at the highest regime temperature of 95 °C. The viscosity curve of extrudate NPPHME from non-treated high moisture feed shows no cold water peak and low viscosity values throughout the pasting cycles. Already from previous assessments (SEI, BD and thermal properties), it has been identified as an extrudate with high levels of non-gelatinised starch and therefore has very limited starch conversion. It could be expected that this non-converted starch would hydrate and impact on the viscosity during the RVA pasting. However, the poor hydration of this material may indicate that the components are unable to swell as they are entrapped in the matrix. It is shown in

**Figure 6-11** that SME is a poor indicator of the cold water and hot peak viscosity and as at a 40 Wh/kg, a very wide range of values were observed.

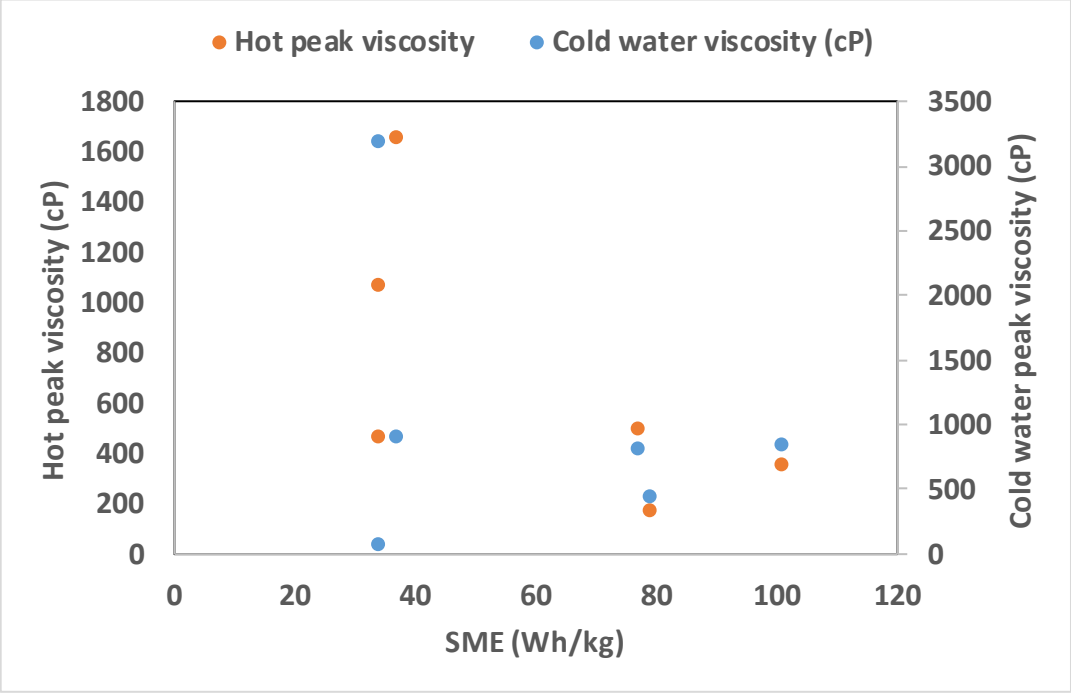



**Figure 6-11:** The effect of SME on cold-water and hot peak viscosities of all the 6 extrudates from treated and non-treated material (this chart was plotted with data from Table 6-4).

Typically, during extrusion the mechanical energy, as defined by the SME, correlates with the conversion undergone by the starch.

Changes to the starch influences the pasting viscosities shown by powdered extrudates when pasted in water. With low SME (<40 Wh/kg), and SEI (1.55), sample HTTHME created using high moisture (22%) in the extruder with the hydrothermally treated feed still shows an increase (two-fold) in cold water viscosity (3195 cP) despite the HTTE having a far greater SME. It does appear that the normal expectation of breakdown of all structures of pigeon pea in the extruder for the samples to be easily hydrated in post extruder was met by HTTHME. The balance for the thermal and shear breakdown of the starch seems to be confounded by the other materials within the cotyledon. These materials in pigeon pea which have undergone hydration and then extrusion seems to be resilient to expansion and hydration. It would be possible to create a product even if it will not be readily expanded, but should show more cold water hydration or viscosity so it would serve well as cold gruel without further heating.

The variations in pasting viscosity of extruded starch reflects the mixtures of raw, gelatinised and degraded starch which co-exists in extruded starch (Gomez and Aguilera, 1984, Lai and Kokini, 1991, Ozcan and Jackson, 2005). As starch granules passes the different stages of extrusion, both in the barrel and in the die, its mode of conversion differs. At low temperature zone of the barrel even with much kneading, native starch may be protected and co-exist with the molten starch. Similar mixtures of fully converted starch can interact with much degraded starch as the SME increases from the shearing process. Therefore, the variations from the co-existence of starch in different structural and molecular levels (native, gelatinised and degraded) affected all the samples pasting behaviour. With this, it can be confirmed that only starch conversion with high cold water viscosity, low hot peak viscosity and higher SME (> 130 Wh/kg) cause an increase in SEI (Figure 6-9) and decrease in the gelatinisation enthalpy (Table 6-5).

### 6.3.6 The textural properties of extrudate from treated feed

The distribution of peak deformation force was used to evaluate the textural properties of the extrudates from treated and non-treated feed. The deformation force measures the force required by the testing probe to penetrate into the interior cells of the extrudate from exterior surface. It corresponds to the force required for the chewing and biting of the products. The highest deformation force measured corresponds to the maximum peak deformation force.

Surprisingly, HTTHME with the highest cold-water viscosity recorded the highest peak deformation force (35.57 N) compared to samples with lower cold-water viscosities. Normally, starch conversion influences viscoelastic properties of the melt and mechanical properties of extrudates. When complete starch conversion (high cold-water viscosity) occurs during extrusion, larger number of air cells are formed. Hence, the deformation force required to puncture the product will be reduced since the cylindrical probe can easily penetrate and disrupt the many air cells. However, with this result of HTTHME, it seems that the type, size and shape of the extrudate cellular structures (cells and cell wall) mostly affects peak deformation force rather than the extent of starch conversion. Earlier, during assessment of HTTHME product physical features, the presence of thick cell walls and coalesced cellular structures were observed.

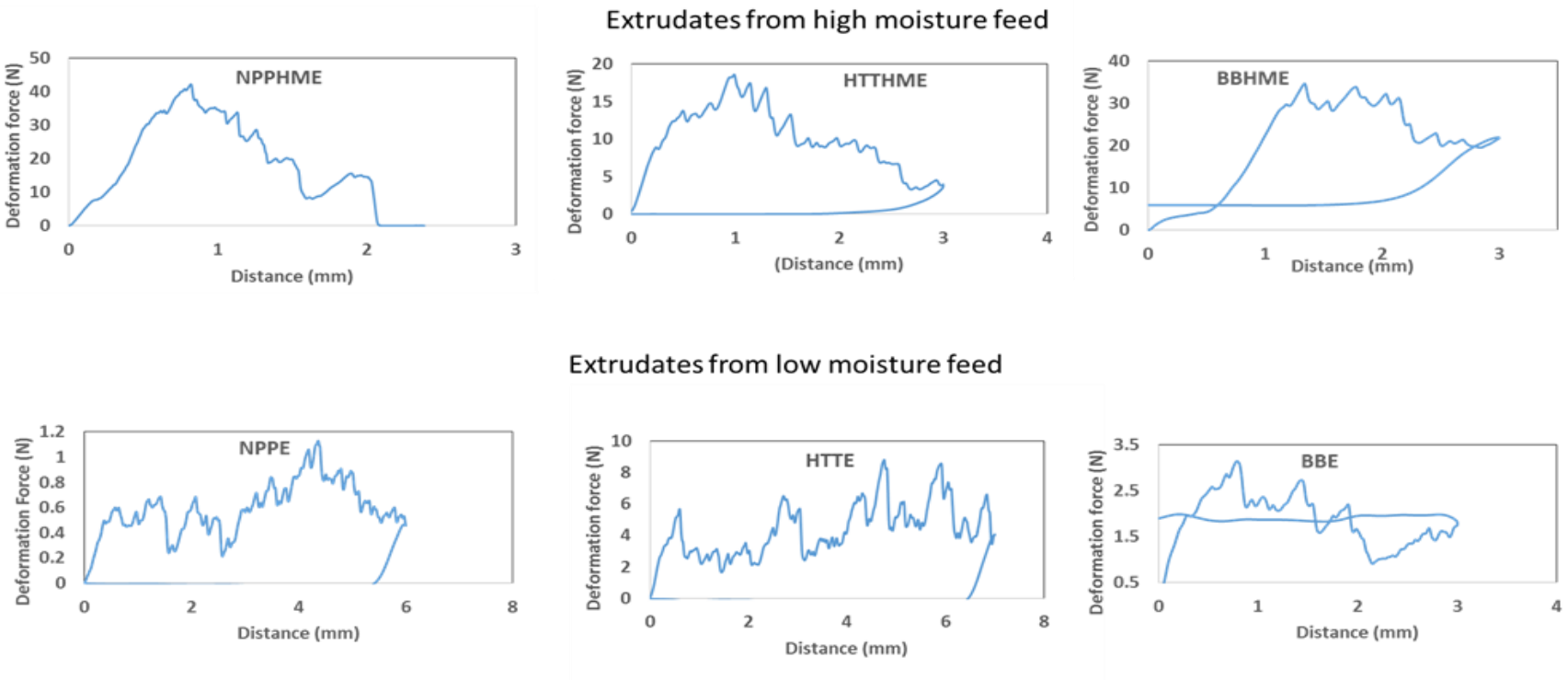



**Figure 6-12:**The distribution pattern of deformation force required for chewing and biting of extrudates from treated and non-treated feeds (measured as the force required to penetrate the extrudate cellular structure with the test cylindrical probe).

MC: Moisture content. NPPHME: extrudate from high moisture (22% MC) native pigeon pea, NPPE: extrudate from (11% MC) native pigeon pea, HTTHME: extrudate from wet (22% MC) milled HTT treated pigeon pea, HTTE: extrudate from dry (11% MC) milled hydrothermally treated pigeon pea, BBHME: extrudate from wet (22% MC) milled alkaline treated pigeon pea, BBE: extrudate from dry (11% MC) milled alkaline treated pigeon pea.

The distance at which the peaks were distributed among the samples differs. In HTTHME, the maximum peak deformation occurred at shorter distance (an indication of hard exterior cellular surface) which corresponds to the peripheral cells or the outer core surface structure of the extrudate while lower deformation peaks were found in the interior (interior is less harder than the exterior). This assumption is based on the texture technique used which records the deformation pattern from the first point (extrudate outer surface) of penetration of the probe into the extrudate until the set distance point (interior cell structure) where the measurement ends. In all the samples (NPPE, HTTHME and BBE) with increased sectional expansion index (SEI), low maximum peak deformation force was noted. This confirms that it is easier to masticate highly expanded porous extrudates. Probably these samples with low deformation peak will make a good quality snacks. The distribution pattern of low moisture expanded extrudates shows that the maximum peak deformation occurred at the middle of the extrudate cross section. Although these extrudates are easy to chew, the core is left with more rigid structures, which adds more stability to the extrudate integrity. All the distribution patterns of the deformation force are evidence of the structural layers that determines the extrudate stability. Generally, extrudate cellular structures emerges after the expansion, and the collapse of the melt following exists from the die.

### 6.3.7 X-Ray diffraction pattern of extrudate starch crystallinity

The X-ray diffraction pattern of the milled powder of extrudates from treated and non-treated feed were determined using wide angle X-ray powder diffractometer (D5005Siemens). The X-ray was generated by copper target and alpha emission (Cu Kα) at 40mA, 40KV while the diffractograms (X-ray diffraction pattern) were obtained from 3-38 thetas (2θ), at 60 rpm spinner stage rotation, and scan rate of 0.05 º (Angle degree step) per 2.5 sec for 29 min.

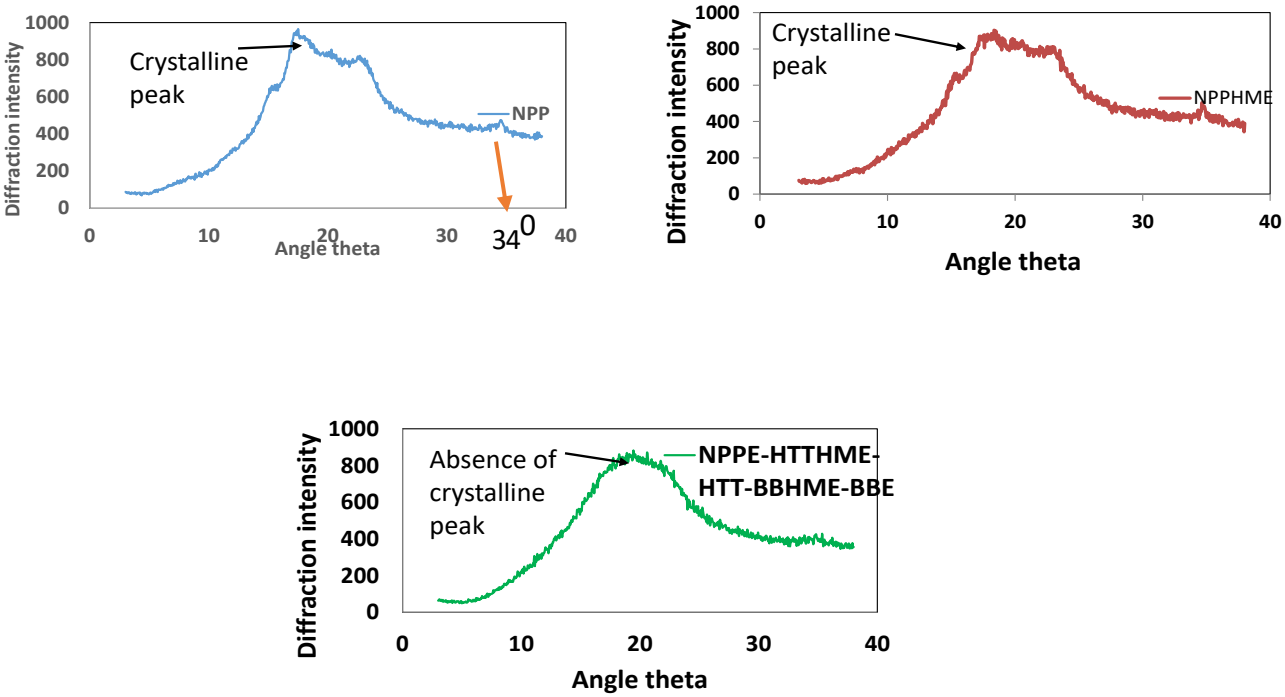



**Figure 6-13: X-**ray diffraction pattern affected by extrusion cooking of treated and non-treated feed material.

Loss of the crystalline peak occurred during extrusion of feed at low moisture.MC: Moisture content. NPPHME: extrudate from high moisture (22% MC) native pigeon pea, NPPE: extrudate from (11% MC) native pigeon pea, HTTHME: extrudate from wet (22% MC) milled HTT treated pigeon pea, HTTE: extrudate from dry (11% MC) milled hydrothermally treated pigeon pea, BBHME: extrudate from wet (22% MC) milled alkaline treated pigeon pea, BBE: extrudate from dry (11% MC) milled alkaline treated pigeon pea.

X-ray diffraction is a reflection of the long order range packing of starch crystallites in a unit cell. This order is altered following the melting of starch crystalline structures. The peaks displayed in the diffractogram indicates the intensity of crystallites relative to the distance between the different crystallites in the sample. The X-ray diffraction pattern **Figure 6-13** of sample NPPHME (half product) shows it retained most of the features of the native NPP X-ray diffraction pattern as most of the starch did not melt during the extrusion process. However, broadening pattern without the usually sharp peak of starch crystalline ordered structure was observed in BBE, BBHME, HTTHME, HTT and NPPE extrudates which earlier shows cold water viscosity. This diffraction pattern featured with broadened peaks are known with V-type starch due to the melting of starch crystallites. Starchy products commonly display the V-type polymorphs after thermomechanical extrusion. The peak at 34º theta (2θ) which was predominant in native and treated pigeon pea were much reduced compared in the extrudate from treated feed material compared to native sample. This peak was proposed to be a globoid crystal of phytin with mineral salts. Fabacaea species to which pigeon pea belongs has been identified with globoids in the protein bodies containing crystals of mineral salt (Lott and Spitzer, 1980, Maenz, 2001, Berrios et al., 2004, Bercu and Razvan, 2015). Despite the absence of X-ray diffraction peak in samples NPPE and HTTE, they still required energy (presence of detectable enthalpy) to melt some order though low for the gelatinisation of the remaining starch granule. It is possible that the new order which require melting results from retrogradation of starch after extrusion and cooling. Retrogradation showed helical order, but not the crystalline alignment necessary for detection by X-ray diffraction. The high SME of these samples during extrusion facilitated the disruption of starch crystalline structure and loss of native X-ray diffraction patterns. Absence of starch crystalline X-ray diffraction peak is one of the product characteristics that confirms that starch conversion has occurred.

### 6.3.8 Evaluation of SEM images of extrudates from treated and non-treated feed material

The evaluation of the microstructural images in

**Figure 6-14** scanning electron microscope (SEM) enhances the understanding of the formation of extrudate from treated and non-treated pigeon pea milled seed powder. The images show that most of the structures found in the native matrix of pigeon pea in Figure 5-11were transformed. As observed in the SEM image in **Figure 6-14**sample NPPHME (extruded from non-treated and high moisture feed) looks like a bulky twisted mass. In addition, some particles of native starch granule were seen distributed in the non-distinguished interior structure confirmed with an absence of voids (air cells). This starch particles were earlier seen on the exterior surface (Figure 6-2) as white patches. The non-separation of the cellular structures into units from the clustered structures suggests that the feed mixture did not melt. This may have resulted in high melt viscosities which prevented bubble nucleation and subsequent growth. Earlier evaluations of NPPHME showing high bulk density, pasting peak viscosity, gelatinisation enthalpy and low sectional expansion index (SEI) were signs

of limited starch gelatinisation. Usually, melting of starch crystalline structure by the combined action of shear and heat reduces viscosity and enhances expansion.

According to literature (Pai et al., 2009, Robin et al., 2011) sectional expansion are more increased when modified bran (high dietary fibre material) were used during extrusion than unmodified (insoluble) bran. The treated bran that increases expansion are known to act as a nucleating agent. Therefore, the non-treatment of NPPHME feed material may have contributed to the limited expansion of the extrudate E9 as most of the fibres remained insoluble and prevents nucleation of bubble.

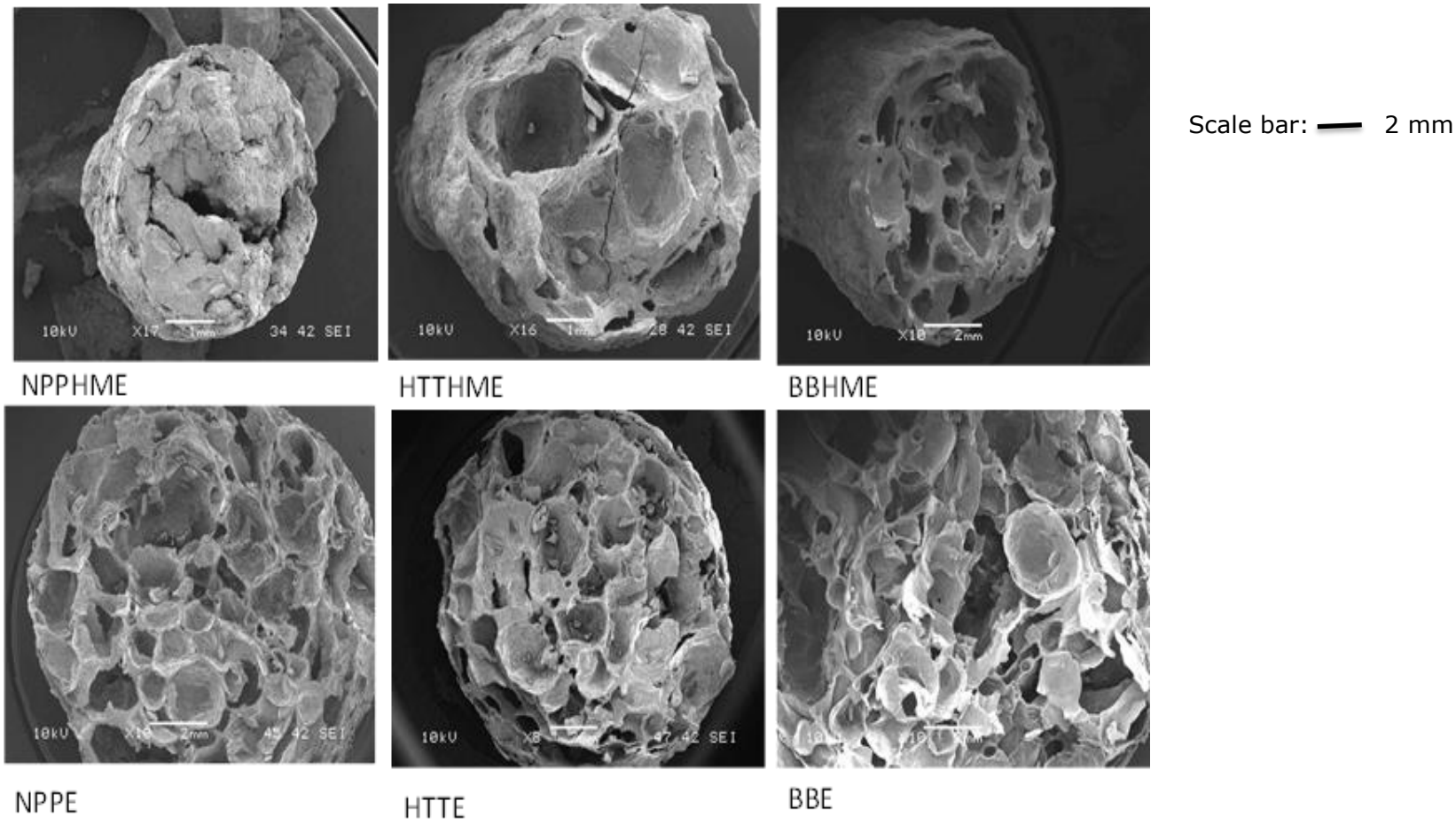



**Figure 6-14:** SEM images of extrudates from treated and non-treated feed extrusion.

The cellular structure of extrudates from low moisture (11%) feed extrusion were filled with more number of air cells which corresponds to an increase in sectional expansion. MC: Moisture content. NPPHME: extrudate from high moisture (22% MC) native pigeon pea, NPPE: extrudate from (11% MC) native pigeon pea, HTTHME: extrudate from wet (22% MC) milled HTT treated pigeon pea, HTTE: extrudate from dry (11% MC) milled hydrothermally treated pigeon pea, BBHME: extrudate from wet (22% MC) milled alkaline treated pigeon pea, BBE: extrudate from dry (11% MC) milled alkaline treated pigeon pea

Both HTTHME and BBHME images show thick cell wall surrounding coalesced closed cells. The few closed cells with large sectional diameter may have contributed to the low sectional expansion index (SEI). In extruded products, coalescing of cells occur when the nucleated bubble integrity is too weak (more viscous) to accommodate the shear stress hence collapse of the bubble wall, merging of cells and reduction in expansion following the sudden disruption of bubble growth. With the fusion of the cells from the sudden rupture of the bubble wall, the cell walls consolidate into a more rigid structure that requires a higher deformation force before it could be masticated. By this cellular structure of HTTME, it has confirmed why high peak deformation force was obtained despite the high cold water viscosity associated with the prior degradation of the native seed cell wall materials and the high starch conversion. Hence the proposal that the viscoelastic properties of the melt has more influence in extrudate cellular structure compared to the starch conversion alone.

Considering the high moisture extrusion of HTTHME and the microstructure, it is possible that a melt with weak integrity and discontinuous network (though initial analysis proved high starch conversion of the product) was formed leading to insufficient solidification of the extrudate after cooling. Consequently, affecting the bubble wall integrity to the collapse of bubble. The collapsed bubble may be joined with another collapsing bubble to form a coalesced cell with larger sectional diameter. Due to lack of sufficient air cells, the porous structure formed by the coalesced cells will have a higher bulk density.

The cellular structures of extrudates from low moisture extrusion (HTTE, BBE and NPP) appeared similar to each other, though BBE differs with more unit air cells. This may indicate the reason for the increased expansion (SEI) and cold water viscosity. The alkaline hydrothermal treatment of BBE feed material may have favoured the formation of more viscoelastic melt capable of higher expansion at die exit. From the observation of the

microstructure, the extrudate was formed from high viscoelastic melt, with self-supporting structure to allow the growth and collapse of the bubble at the appropriate time to have the more air cells and the higher SEI. As the exit melt cools rapidly due to the loss of moisture content, it will cause the bubble to stabilise. In contrast to BBHME and HTTHME, the porosity of NPPE, HTTE and BBHME resulted in lower bulk density and high SEI because of the high number of air cells. Much degradation of the cell walls materials after the alkaline treatment favours creation of more air cells in BBE and the formation of more viscoelastic melt that is capable of expansion by die swell.

### 6.3.9 Fluorescence imaging of extrudate cellular components

The cellular structure of extrudates' major chemical components (starch, protein and fibre) were observed using wide field epifluorescence microscope (DMRB, Leica Microsystems Wetzlar, Germany). Three channels corresponding to the three fluorescent dyes (FITC, rhodamine B and calcolfluor white) were used intermittently to label these components in the extrudate for a simultaneous viewing. The fluorescence emitted were merged with the image, dye colour and channels. Full details of the methodology are given in section 3.10.4. The list of extrudate components and corresponding fluorescence dyes are available in Table **6.6-6**. The fluorescence images in Figure 6-15 to Figure 6-20 shows some variations due to the interactions among the extrudate components.

**Table 6.6-6:** Dye and colours of the extrudate cellular components.

| Components | Starch | Protein | Fibre |
|---|---|---|---|
| Fluorescence dye | FITC | Rhodamine B | Calcofluor |
| Fluorescence channel colours of component image | Green | Red | Blue |

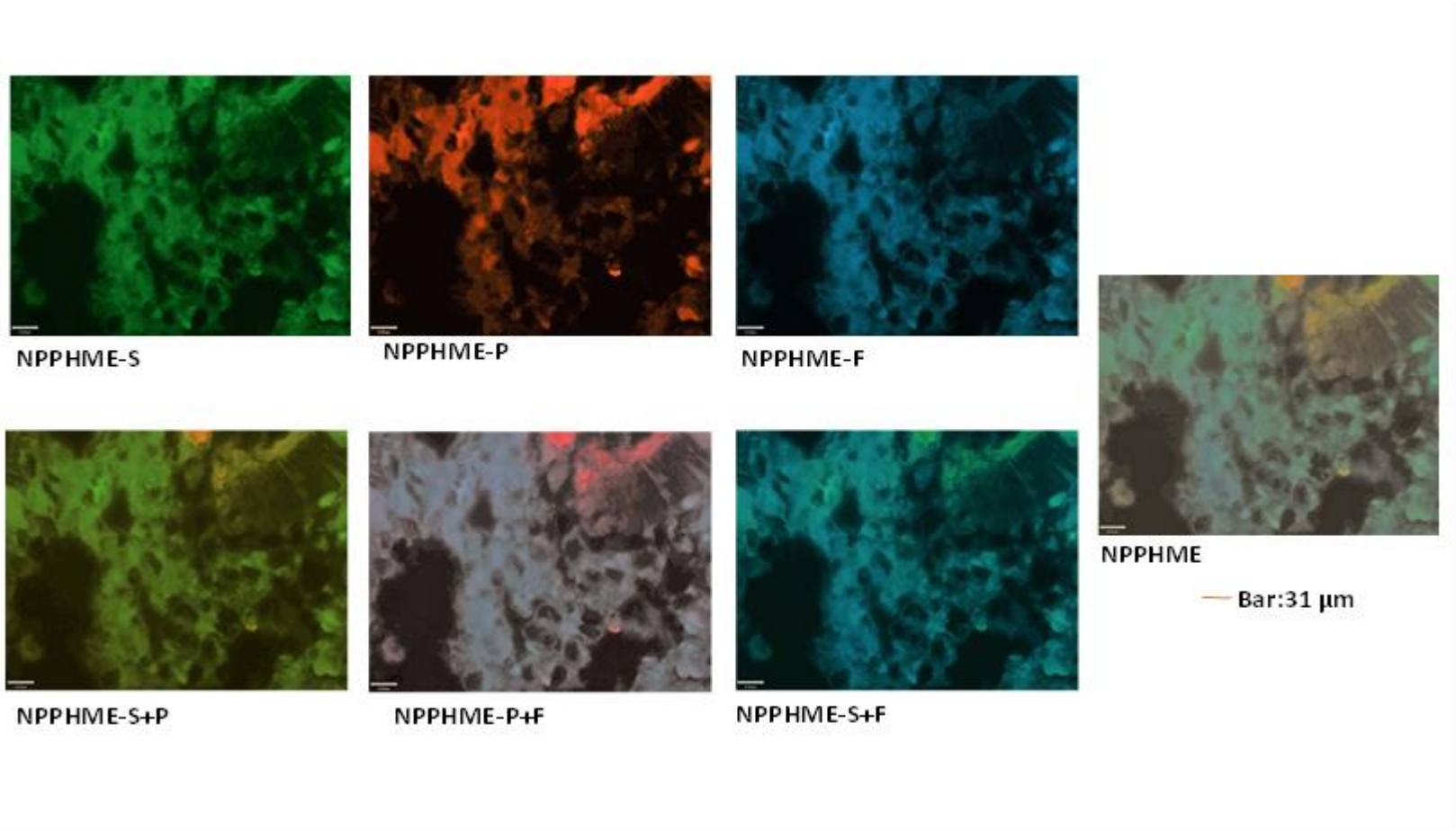



**Figure 6-15:** Fluorescence images of cellular components in NPPHME.

S: Starch, P: Protein, F: Fibre. NPPHME: extrudate from high moisture (22%) native pigeon pea and extrusion conditions of screw Speed: 400RPM, die temperature 150 (°C) and feed rate 14 kg/h.

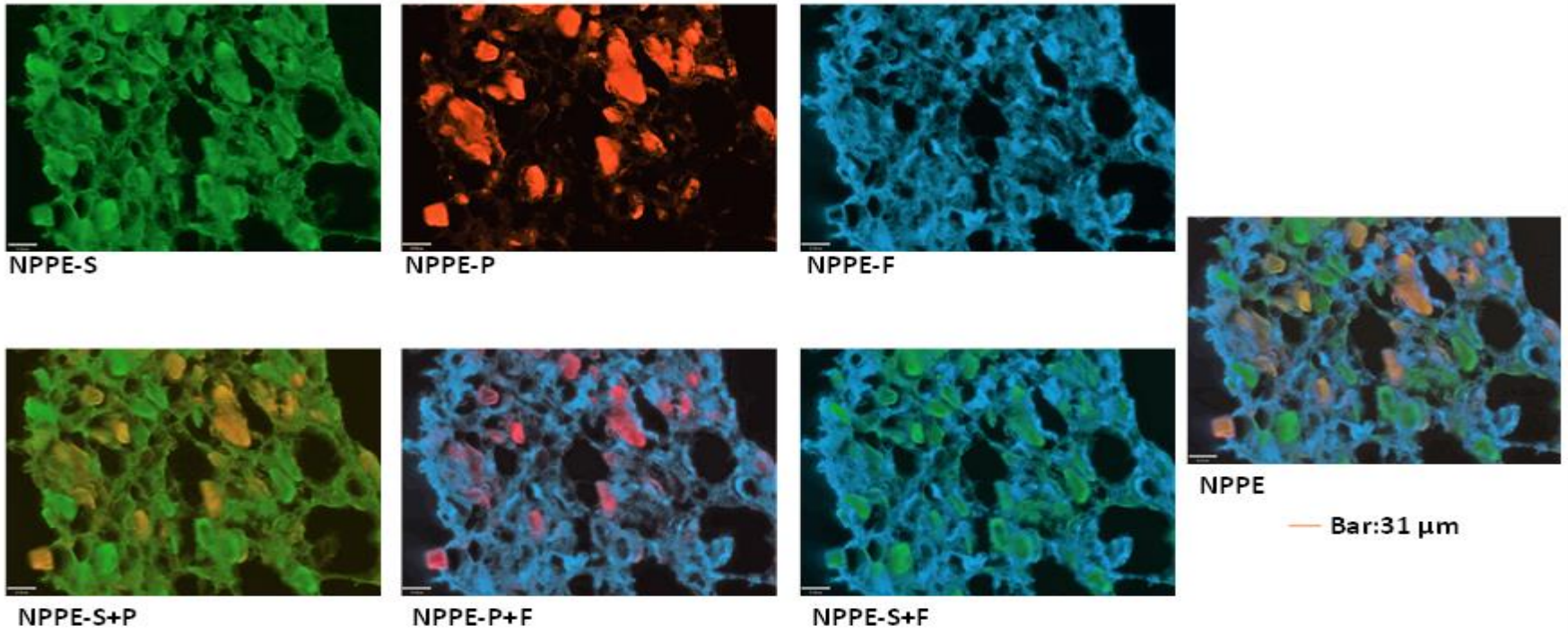



**Figure 6-16:** Fluorescence images of cellular components in NPPE.

S: Starch, P: Protein, F: Fibre. NPPE: extrudate from low moisture (11%) native pigeon pea and extrusion conditions: screw Speed: 400 RPM, die temperature: 150 °C and feed rate: 14 kg/h.

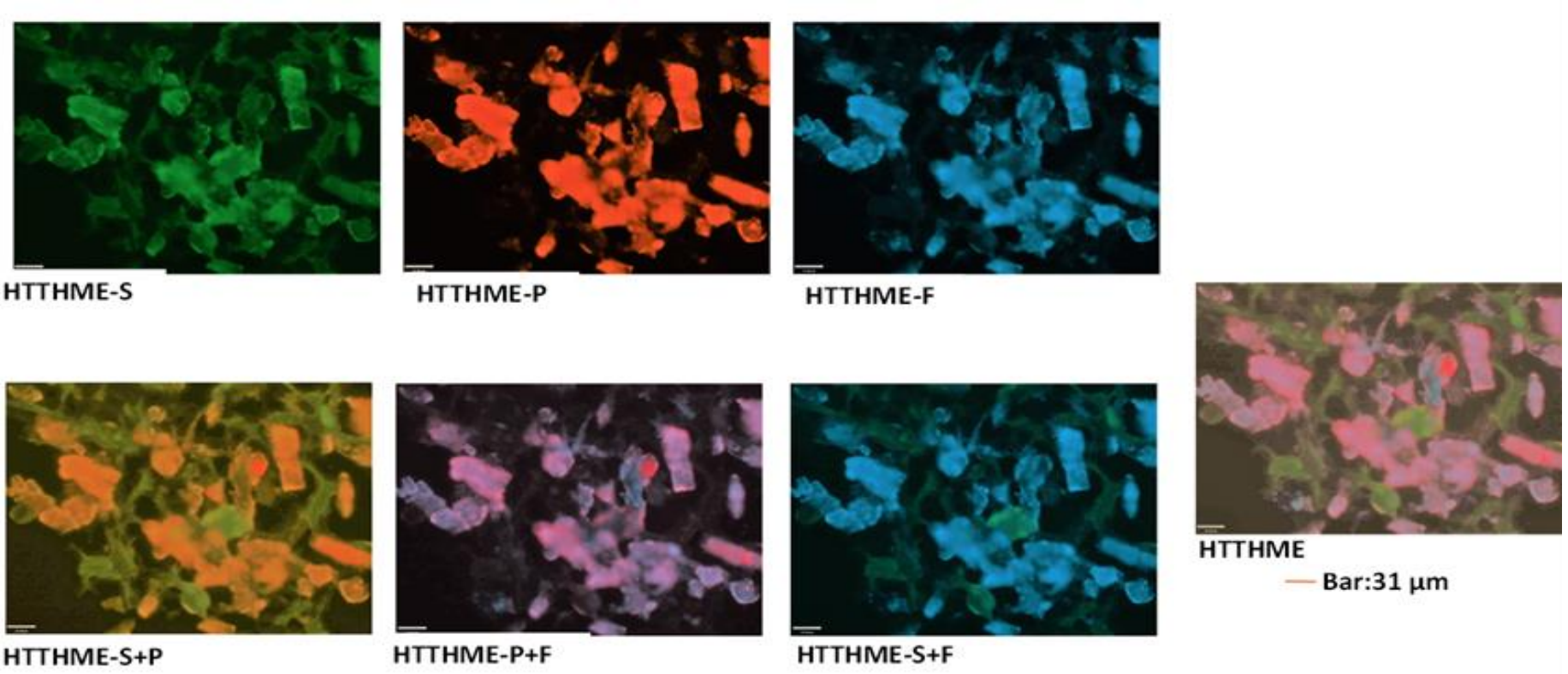



**Figure 6-17:** Fluorescence images of cellular components of HTTHME.

S: Starch, P: Protein, F: Fibre. HTTHME: extrudate from wet milled (22% moisture content) hydrothermally treated pigeon pea and extrusion conditions: screw Speed: 400 RPM, die temperature: 150 °C and feed rate: 14 kg/h.

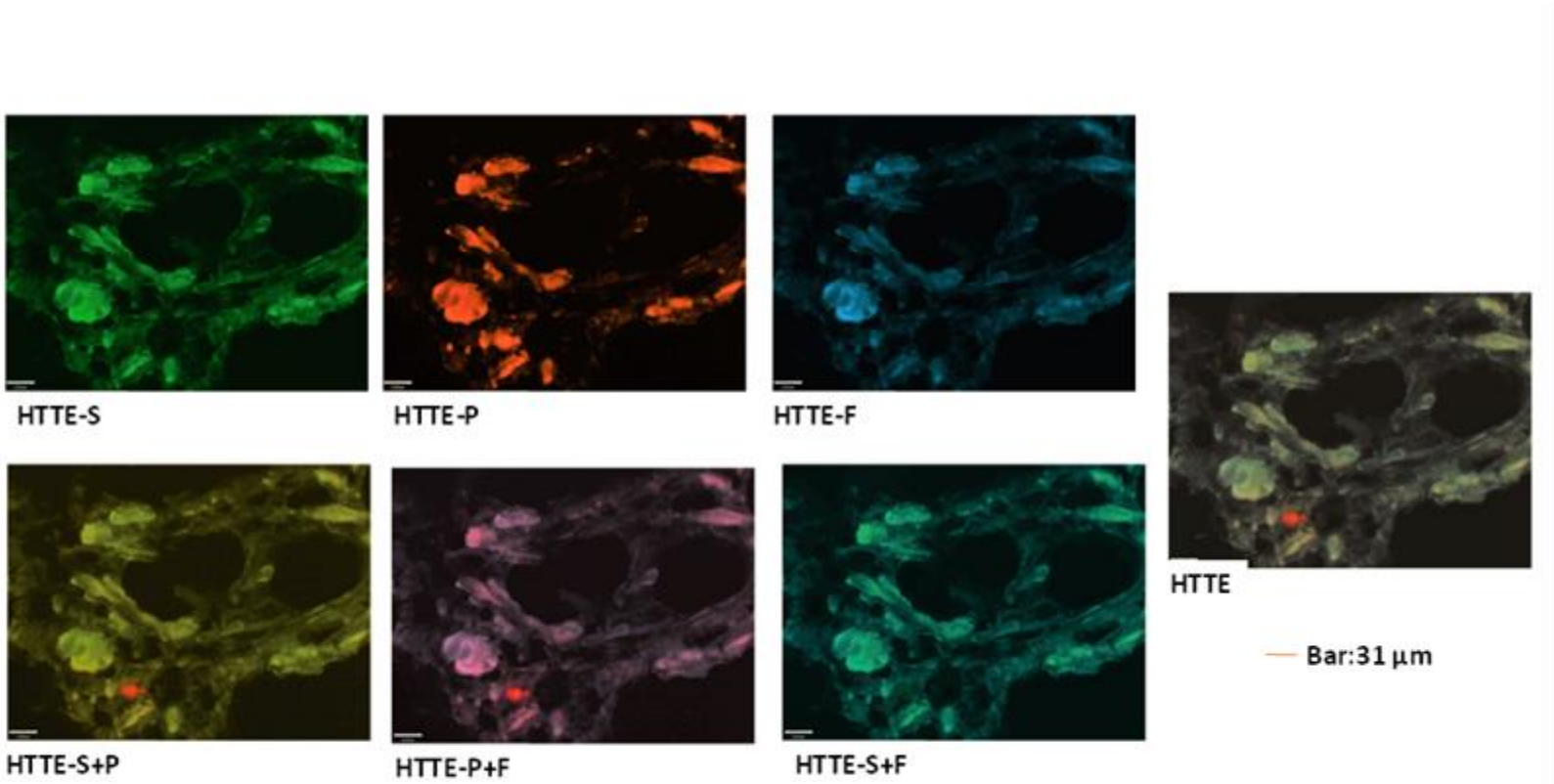
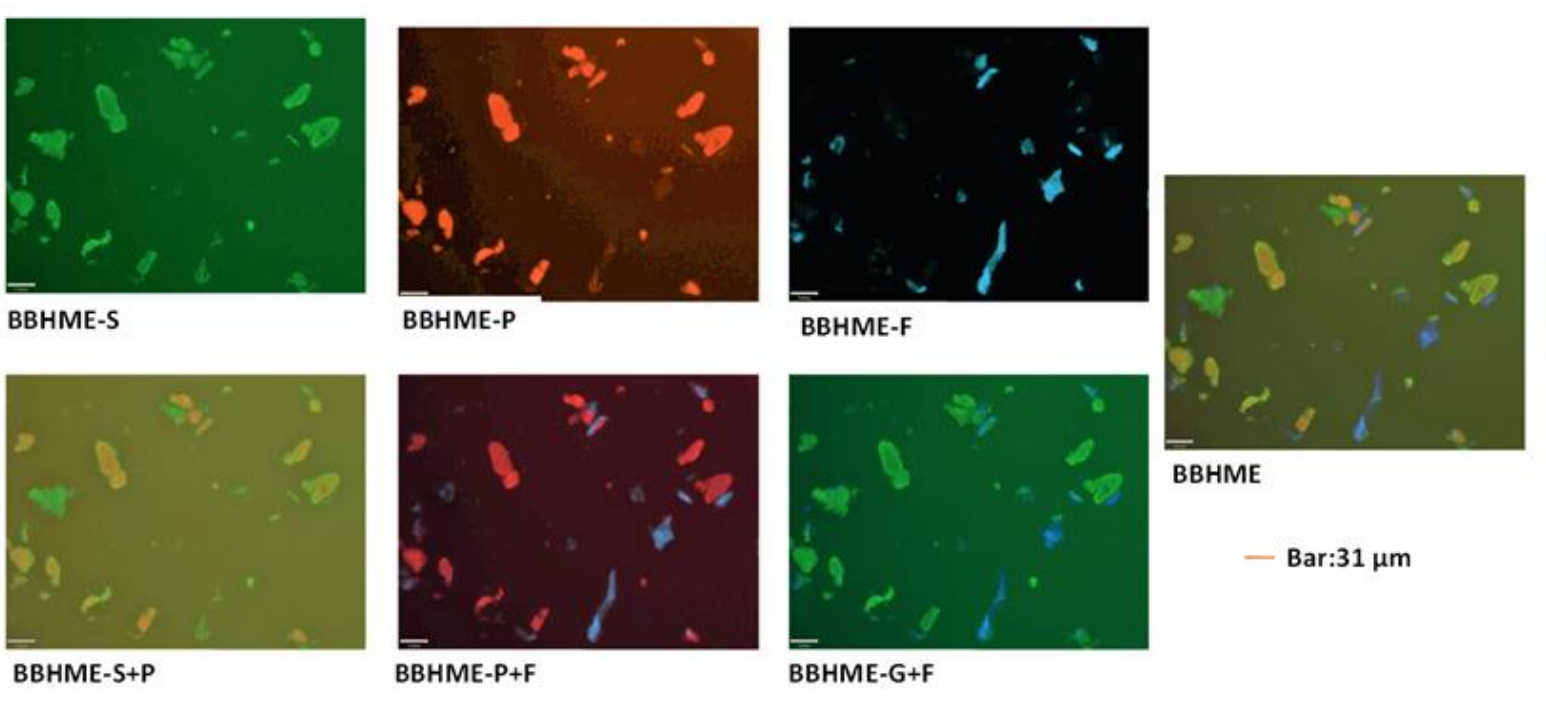



**Figure 6-18:** Fluorescence images of cellular components in HTTE.

S: Starch, P: Protein, F: Fibre. HTTE: extrudate from dry milled hydrothermally treated pigeon peaand extrusion condition: screw Speed: 400 RPM, die temperature: 150 °C and feed rate: 14 kg/h.

**Figure 6-19:** Fluorescence images of cellular components in BBHME.




S: Starch, P: Protein, F: Fibre. BBHME: extrudate from wet milled alkaline treated pigeon pea, and extrusion conditions: screw Speed: 400 RPM, die temperature 150 °C and feed rate: 14 kg/h.

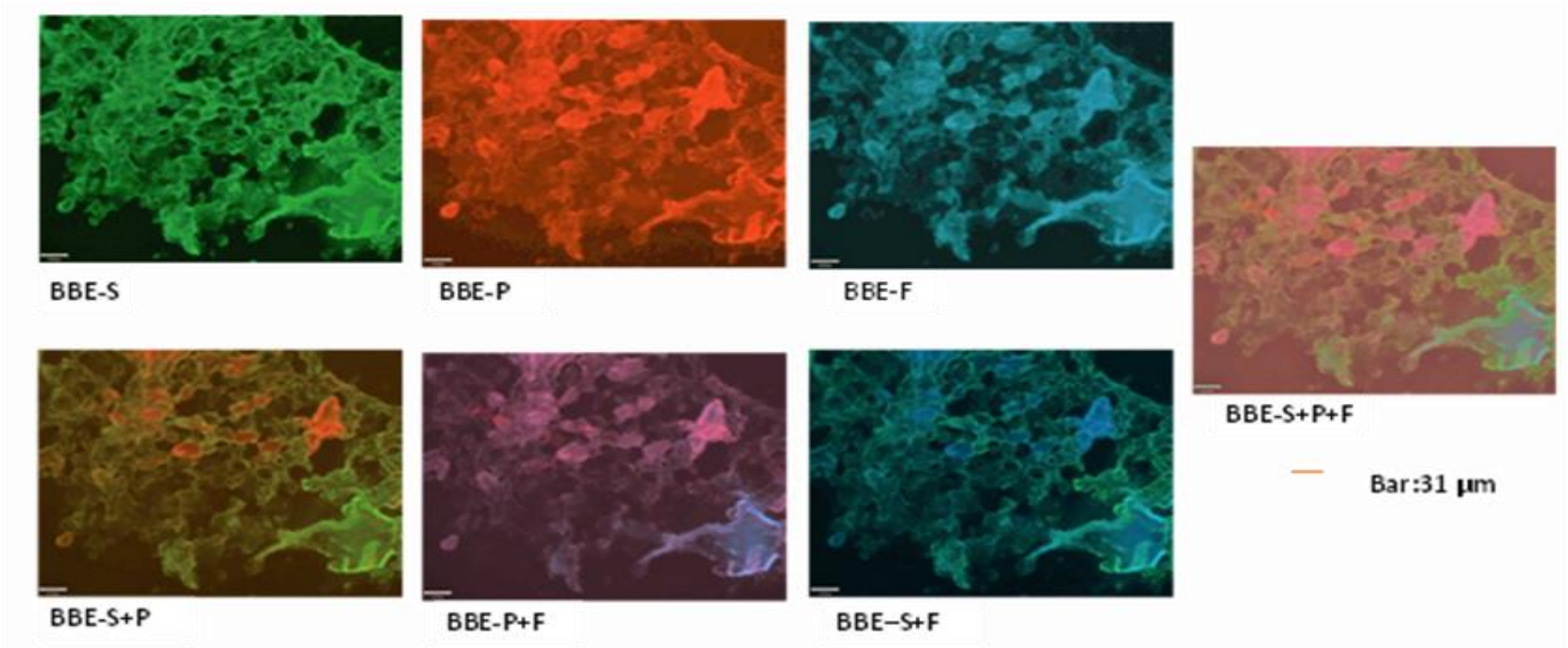



**Figure 6-20:** Fluorescence images of cellular components in BBE.

S: Starch, P: Protein, F: Fibre. BBE: extrudate from dry milled alkaline treated pigeon pea, and extrusion conditions: screw Speed: 400 RPM, die temperature: 150 °C and feed rate 14 kg/h.

#### 6.3.9.1 NPPHME fluorescence images

In view of the non-treatment and high moisture content of NPPHME feed material, the kneading of the feed during the extrusion resulted in the structural components forming a complex massed microstructure without clear distinction of cellular units in Figure 6-15. The absence of distinguished unit cells may not mean that the cellular components are not segregated. The consequence of limited shearing of the feed material due to the high moisture content may mean that the components were still within the cell. In addition, the particulate nature of these components hinders the formation of viscoelastic melt necessary for expansion and formation of unit cells. Hence the poorly expanded hard product was formed. Previous evaluation confirmed the occurrence of limited (almost absence) starch conversion during formation of NPPHME. In addition, the SEM image in Figure 6-8 shows some granules in the native state.

#### 6.3.9.2 NPPE fluorescence images

However, in extrusion of similar material with NPPHME but at low moisture there was an incomplete starch conversion (NPPE) which affected the interaction of starch with other cellular components. Though the protein aggregates were distributed as discrete particles but its attachment on the starch-fibre continuous network may have resulted in the increased expansion of NPPE when compared to NPPHME.

#### 6.3.9.3 HTTHME fluorescence images

Surprisingly, the protein and fibre of HTTHME (Figure 6-17) from high moisture hydrothermally treated feed interacted very well without much starch involvement. The sample was earlier identified with highest cold water viscosity, an indication of high level of starch gelatinisation. This interaction between protein and fibre were lacking in BBHME produced with alkaline hydrothermally treated feed material at high moisture content. However, in HTTHME less structural stability occurred as the starch did not form continuous network with protein and fibre. The reason for this starch

behaviour may be attributed to the collapse of bubbles from weak viscoelastic melt resulting in coalescing of the cells. Since the starch did not associate with protein and fibre, then protein and fibre may have been a reinforcement stabilising the extrudate cellular structure.

#### 6.3.9.4 HTTE fluorescence images

In **Figure 6-18**a high level of interactions of all the chemical components existed. Evidence as the overlays channel image shows a distinct colour of continuous network without presence of individual fluorescence. Since the SEM image of the sample shows well distributed air cells, then it can be confirmed that the mutual interactions of all components occurred when there were sufficient melted molten starch and degraded non-starch components.

#### 6.3.9.5 BBHME fluorescence images

In BBHME (Figure 6-19), more dispersion of all the components occurred without any continuous matrix though interaction of protein and starch predominate with intermediate linkages provided by the fibre particles. Though the alkaline treatment facilitated the degradation and solubilisation of the fibre but because of the high moisture extrusion that reduced the frictional force, less starch conversion occurred which affected formation of homogenous and starch continuous melt. This limited interaction may have affected the viscoelasticity of the melt, stability of the bubble walls and consequently limiting expansion of the bubble (low SEI).

#### 6.3.9.6 BBE fluorescence images

Although, in BBE (Figure 6-20)perfect interactions seems to have existed between protein, starch and fibre but still some protein separation occurred towards the edge of the exrudate cells. The separated protein components though denatured (high fluorescence intensity), but due to self-cross-linked (conjugated protein) did not interact with the starch and fibre network. At the point where, the protein structure discontinued with the continuous

extrudate network, the fibre structures appeared to be more embedded and superimposed on the starch continuous matrix. It is possible that some segregation of the protein may occur at the cooling and drying of the extrudates as well as during retrogradation. Through their separation from the continuous matrix of starch-fibre, it assembled towards the edge of the air cells. Already it has shown in HTTE that fibres (cell wall materials) from treated feed material forms continuous matrix with starch.

#### 6.3.9.7 Summary of fluorescence cellular images

The fluorescence images of the extrudate cellular structure have shown that expansion of extrudate occurs when the deformed starch granules from high starch conversion predominates extrudate continuous matrix. In addition, the compatibility of starch with the non-starch components of the melt forms a homogenous network which is the foundation for the formation of stable ready to eat expanded extrudate. In some extrudates, due to the less degradation of the native structure, limited interactions and compatibility occurs among the cellular components leading to phase separation. The fluorescence images are another evidence that shows that due to very low shear viscosity of the high starch conversion melt from high moisture extrusion (BBHME and HTTHME), the non-starch components form a different network. The separation of the non-starch components from starch continuous matrix results in sudden rupture of the bubble and coalescing of cells. Hence confirming that the degraded non-starch components confers stability (reinforcement) to starch network and where they separate from starch network, the extrudate is less stable as in NPPHME, where the extrusion cooking conditions could not transform the materials' native matrices for the formation of elastic melt, air cells were not formed.

Earlier report on NPPE shows insufficient melting of crystalline structure (low cold water viscosity and increased hot paste peak viscosity and gelatinisation enthalpy) which may be due to the less availability of water

to starch from the non-treatment of the feed. Therefore, this might have been reflected on the fluorescence image which shows the protein aggregates were distributed as large particles on the starch-fibre continuous network and lowest deformation force (2.1 N) recorded.

## 6.4 Chapter conclusion

In addition to poor quality (physical features) of some of the extrudate from high moisture extrusion, the expansion properties (bulk density and sectional expansion index) were not satisfying. These extrudates produced from high moisture feed developed some low cold water peak viscosity, which is considered evidence of low starch conversion. The lack of starch conversion could have been due to the low frictional forces exhibited in the barrel of the extruder from the high moisture feed material. The extrusion responses also show that at higher moisture content and feed rate, the torque required to rotate the screw and the feed decreases, thereby resulting in low SME and poor quality product. At the higher temperatures of 140 °C to 150 °C and screw speed of 300-400 rpm, the expansion characteristics of the extrudates (E6, E8 and E9), as indicated by lower bulk densities and higher expansion indices, were improved. For direct expansion to occur it is necessary to have the starch as the continuous matrix so that bubble nucleation and growth could occur.

The preliminary extrusion at low moisture feed also established that native pigeon pea powder could be extruded into stable directly expanded product. But to optimise pigeon pea extrusion cooking and obtain more stable products, the thermomechanical extrusion was best carried out with low moisture (11%) feed material, die temperature of 150 °C, screw speed of 400 rpm and feed rate of 14 kg/h. With these conditions, the expansion properties of extrudate from treated feed improved compared to extrudates from non-treated feed. As a good indication that less force is required during chewing/biting of this extrudate, a low deformation peak force was obtained when puncturing these extrudates (NPPE, HTTE, and BBE) from

low moisture feed. At the low moisture extrusion, especially as it pertains to treated feeds, the more the extrusion cooking solubilises and degrades the melt components (starch, protein and fibre), the more the extrudate WSI increased. The low bulk density of BBE would offers both the manufacturer and consumers the convenience in handling.

Interestingly, no gelatinisation enthalpy (high melting of native starch structure) was noted on extrudates BBE and BBHME produced from the alkaline treated feed. Since all the extrudates from the low (11%) moisture treated feed material exhibited no crystalline peak in the X-ray diffraction pattern (an indication of disruption of starch crystalline structure), the hydration treatment could be a step in the optimisation of pigeon pea extrusion. The SEM images of extrudates from the higher feed (14kg/h) and low (11%) moisture extrusion, shows that the higher the number of air cells, the more the expansion of the extrudates. Surprisingly, an observation of the BBE fluorescence image suggested some protein molecules were not part of a continuous network of starch and fibre. This phase separation of protein was attributed to protein changes in the extruder occurring at the same time as the starch conversion. The segregation of the denatured proteins may occur during the cooling and drying phase of the extrudates' manufacture. Furthermore, fluorescence image shows that expansion mostly occurs in extrudates from low moisture extrusion where the extrudate's starch continuous network originated from high starch conversion and formation of a melt, in addition to mutual interactions between starch and non-starch components (protein and fibre). The subsequent chapter will focus on the nutritional implications of the extruded Pigeon pea to ascertain if the consumption of this product would be safe.

# 7 The nutritional qualities of the extrudates

## 7.1 Introduction

The determination of the nutritional and anti-nutritional composition of extrudates from treated and non-treated feed was necessary, as the majority of the extrudates are ready to eat snack food. Conventionally, in developing nations, pigeon pea is consumed as a major source of protein and importantly as an alternative to the costly animal protein. Considering the chemical composition of the non-extruded powdered pigeon pea in chapter 4, the extrudates could be a good source of food for the food security of developing nations and the world. However, as the nutritional quality of food depends majorily on the availability of nutrient after ingestion, of concern was that pigeon pea contained some anti-nutritional factors which may interfere with digestion and nutrition (Onwuka, 2006). Reduction through thermomechanical extrusion has also been recommended as a way of removing anti-nutritional materials. More information from literature on nutritional and anti-nutritional composition of pigeon pea are available in section 2.2.2 respectively.

Although functional, thermal and structural characteristics were assessed to define the extrudate qualities, information on nutritional and anti-nutritional composition would also be required for health and safe consumption. Therefore, the major nutritional composition (protein, starch and fibre) and some anti-nutritional factors (phytic acid, oxalate and trypsin inhibitor) of the extrudates were evaluated. The evaluation was according to methods available in section 3.4.6 (nutritional composition) and 3.5 (anti-nutritional factors). The Anti nutritional factors (ANF) analyses were performed with the milled powders obtained from the three most stable extrudates of treated (HTTE and BBE) and non-treated (NPPE) low moisture feed materials. The results were compared with the non-extruded sample (NPP) to ascertain the impact of the thermomechanical extrusion.

## 7.2 Impact of extrusion on extrudates' nutritional composition

The protein contents of the milled extrudates (Table *7-1*) were analysed using the Dumas method (section 3.4.6.4). The result shows no significant difference in the protein content between any of the samples. Both the non-extruded sample and extrudates show an average protein content of 21% which is within the values reported in the literature (Fasoyiro et al., 2010, Saxena et al., 2010). Based on this average protein content, the extrudates would be a good source of protein to the consumers. However, the protein digestibility determined with multi-enzyme assay (section 3.4.6.5) were significantly different at $p<0.05$ with a higher level of digestibility for the extruded samples. Analytically, the protein digestibility is described as the percentage of the protein content that is efficiently digestible and available for human nutrition. It depends on the extent of protein structural modifications during denaturation, aggregation and cross linking. Protein in a completely denatured state seems to exhibit higher digestibility if there is no inclusion of anti-nutritional factors that inhibits protein digestive enzymes. Except for the native pigeon pea (NPP) with the lowest protein digestibility of 65%, all the extrudates show a high potential for protein digestion (Alonso et al., 2000a).

**Table 7-1: Nutritional composition of extrudates from treated and non-treated feed material.**

| | Protein content | Protein digestibility (%) | Total dietary fibre (%) | Insoluble dietary fibre (%) | Soluble dietary fibre (%) | Starch content (%) | Starch digestibility (%) |
|---|---|---|---|---|---|---|---|
| **NPP** | 22.57±0.24$^{a}$ | 65.17±0.91$^{a}$ | 28.05±1.01$^{d}$ | 23.67±1.06$^{c}$ | 4.38±2.12$^{a}$ | 39.26±2.59$^{b}$ | 16.53±1.20$^{a}$ |
| **NPPHME** | 22.41±0.75$^{a}$ | 69.58±0.89$^{b}$ | 25.33±0.48$^{cd}$ | 21.36±0.60$^{bc}$ | 3.97±0.12$^{a}$ | 33.76±1.11$^{a}$ | 90.48±0.25$^{b}$ |
| **NPPE** | 20.36±0.86$^{a}$ | 70.30±0.89$^{b}$ | 23.60±0.73$^{bc}$ | 17.06±0.45$^{ab}$ | 6.53±0.28$^{a}$ | 33.98±0.19$^{a}$ | 96.24±0.45$^{c}$ |
| **HTTHME** | 21.31±0.97$^{a}$ | 70.67±1.06$^{b}$ | 22.09±0.13$^{ab}$ | 17.92±1.09$^{ab}$ | 4.17±1.22$^{a}$ | 36.29±2.4$^{ab}$ | 98.69±0.11$^{de}$ |
| **HTTE** | 22.06±0.95$^{a}$ | 72.41±0.75$^{bc}$ | 25.37±0.82$^{cd}$ | 19.00±2.46$^{abc}$ | 6.36±1.64$^{a}$ | 37.61±1.89$^{ab}$ | 97.16±0.88$^{c}$ |
| **BBHME** | 23.08±0.00$^{a}$ | 74.58±2.27$^{c}$ | 23.12±0.76$^{bc}$ | 16.33±0.94$^{a}$ | 6.78±1.70$^{a}$ | 35.62±1.77$^{ab}$ | 99.63±0.08$^{e}$ |
| **BBE** | 22.10±0.25$^{a}$ | 71.03±0.99$^{b}$ | 19.88±0.96$^{a}$ | 17.57±0.12$^{ab}$ | 2.31±1.09$^{a}$ | 37.73±0.54$^{ab}$ | 97.16±0.28$^{cd}$ |

Values are mean of triplicate with ± standard deviation. The mean with different letters in the same column are significantly different ($P<0.05$) as per Tukey multiple comparison calculated by difference.

Although the protein digestibility for the extrudates were significantly different from the native ground pigeon pea there was no trend in distinguishing between the extrudates for their protein digestibility. It could be difficult to explain the variations in the protein digestibility among the extrudates since no other evaluation in this study was used to investigate the actual state of protein (degree of denaturation or aggregation) after the thermomechanical extrusion. Therefore, it can be suggested that either more denaturation or deactivation of anti-nutritional factors occurred during the extrusion of BBHME with highest protein digestibility.

Presently, much awareness has been raised on the importance of dietary fibre in human nutrition and wellbeing. Dietary fibre has been defined as plant materials which cannot be digested by the human digestive enzymes. They include cellulose, hemicellulose, pectin, gum, waxes, lignin and beta-glucan. Usually sub-divided into soluble and insoluble dietary fibre. Lots of health benefits have been attached to the consumption of dietary fibre. These includes reduction of blood cholesterol, constipation and heart related diseases (Khan et al., 2007). Based on these health benefits, the powder from whole seed was used in the extrusion instead of the dehulled seed flour as much fibre are available in the seed coat. The assay used in determination of the total fibre is available in section 3.4.9.

From Table 7-1, the total dietary fibre of all the extrudates ranged from 19.88% to 25.37% which is higher than what was reported(Khan et al., 2007, Vasishtha et al., 2012). These levels of fibre could contribute to an adult recommended daily intake of 18g. With these fibre levels, the extruded products are high fibre snack food since the fibre content is more than 6g/100g which is the approved standard for high fibre food products (BNF, 2017) Due to degradation during extrusion a reduction (4% to 24% loss) of total dietary fibre occurred. This loss was more pronounced in BBE while HTTE had the least impact. However, the data in Table 7-1 shows that extrusion cooking through the degradation of the insoluble fraction,

increased the soluble fibres of the extrudates. Further loss of total fibre can also occur through the solubilisation and leaching of soluble fibre especially the pectin components, which is easily degradable by thermal decomposition through beta elimination (section 2.3.2).

Modification of starch granule through extrusion cooking has effect on both starch concentration (starch content) and digestibility. Reduction of starch content would be apparent if encapsulation or degradation (lower molecular weight polymers) occurred during extrusion. Although the Megazyme starch assay procedure relies on starch digestive enzymes' ($\alpha$-amylase amyloglucosidase) hydrolysis action on the milled extrudates' starch, it may be difficult to determine the total starch content if the starch granule were encapsulated in the native structures within the cells or if this encapsulation were not disrupted during the extrusion.

In order to ascertain the availability of extrudate starch following digestion, starch digestibility was measured with Megazyme assay procedure (section 3.4.6.2). The starch digestibility represents the percentage of the starch content that would be capable of digestion by the human digestive enzymes after consumption. The conversion, gelatinisation and exposure of starch following extrusion determines the extent of digestibility. Only higher starch conversion would result in higher digestibility provided the starch were not compartmentalised. The low starch digestibility of non-extruded native pigeon pea (NNP) is evidence of non-gelatinisation of the starch. The starch digestibility also reduced in NPPHME, already assessed with low starch conversion and presence of native starch granule. With the increase in starch digestibility of most of extrudates with high starch conversion, energy associated with human starch digestion can be easily utilised in various physiological activities.

## 7.3 Amino acid profile of the extrudates.

The amino acid profile of the extrudates were investigated as it is important in human nutrition. By definition, the amino acids are organic substances required for body growth and maintenance. Among the amino acids are some known as essential amino acid that the body cannot synthesize. As displayed in Table 7-3, the extrudates contained substantial amino acids as analysed with oxidative and hydrolysis method (section 3.4.6.5). These levels of amino acid can meet the FAO and recommended daily intake if consumption level was monitored (Apata and Ologhobo, 1994).

**Table 7-2:** The abbreviation and full name of the amino acids.

| Abbreviation | Full name of amino acid | Abbreviation | Full name of amino acid |
|---|---|---|---|
| Cys | Cysteine | Ile | Isoleucine |
| Asp | Aspartate | Leu | Leucine |
| Met | Methionine | Tyr | Tyrosine |
| Thr | Threonine | Phe | Phenylalanine |
| Ser | Serine | Lys | Lysine |
| Glu | Glutamate | His | Histidine |
| Gly | Glycine | Arg | Arginine |
| Ala | Alanine | Pro | Proline |
| Val | Valine | | |

**Table 7-3**: Amino acid (g/kg sample) profile of the extrudates. Values represents the average of replicate analysis

| Sample Code | Cys | Asp | Met | Thr | Ser | Glu | Gly | Ala | Val | Ile | Leu | Tyr | Phe | Lys | His | Arg | Pro |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NPP | 2.8 | 21.42 | 2.17 | 7.1 | 9.24 | 39.73 | 7.16 | 8.77 | 8.92 | 7.6 | 14.7 | 4.23 | 21.94 | 14.04 | 7.18 | 12.1 | 9.52 |
| NPPHME | 2.5 | 21.53 | 2.00 | 7.11 | 8.7 | 39.89 | 7.33 | 9.02 | 8.9 | 7.78 | 15.18 | 4.04 | 21.95 | 14.39 | 7.38 | 13 | 9.08 |
| NPPE | 2.53 | 20.81 | 2.07 | 6.78 | 9.29 | 40.64 | 7.05 | 8.51 | 8.5 | 7.44 | 14.94 | 4.34 | 21.96 | 13.68 | 7.18 | 12.36 | 8.85 |
| HTTHME | 2.27 | 14.76 | 1.53 | 5.1 | 6.53 | 28.71 | 5.45 | 6.95 | 7.18 | 5.77 | 10.8 | 3.51 | 15.01 | 9.87 | 5.47 | 8.88 | 6.66 |
| HTTE | 2.19 | 14.81 | 1.74 | 5.12 | 6.47 | 29.45 | 5.46 | 6.62 | 7.22 | 5.96 | 10.93 | 3.31 | 15.95 | 9.53 | 5.43 | 8.73 | 6.65 |
| BBHME | 2.37 | 20.24 | 1.67 | 6.71 | 8.82 | 38.46 | 6.83 | 8.49 | 8.59 | 7.36 | 14.52 | 4.27 | 20.76 | 13.67 | 6.88 | 12.22 | 8.2 |
| BBE | 2.39 | 21.28 | 1.95 | 6.92 | 9.08 | 39.78 | 7.37 | 8.93 | 9.12 | 8.13 | 15.41 | 4.92 | 21.37 | 13.19 | 7.66 | 12.43 | 9.89 |

It was noted that seven out of the eight essential amino acids (valine leucine, lysine, threonine, histidine, isoleucine, methionine and tryptophan) were available in pigeon pea extrudates although tryptophan was not detected. Essential amino acids are group of amino acids required by the body which the body cannot produce. The essential amino acid obtained in greatest quantity was leucine, while methionine, which is rich in sulphur amino acid, was limiting. Generally, legumes contain insufficient sulphur amino acids. Glutamate was the most prevalent amino acid detected. Similar to other legumes, the level of lysine was high in the native Pigeon pea material. High levels were maintained in the extruded pigeon pea and there was more than reported for cereals where insufficient lysine has been recorded (Apata and Ologhobo, 1994, Ruiz-Ruiz et al., 2008).

Due to extrusion cooking, certain amino acids were either lost or gained. The variations in the level of amino acids may depend on the same factors (degree of denaturation, aggregation and deactivation of anti-nutritional factors) earlier mentioned that affected protein digestibility. The amount of amino acids detected in this study corresponded with other authors evaluations of native pigeon pea (Singh et al., 1980, Singh et al., 1990, Apata and Ologhobo, 1994).

## 7.4 The anti-nutritional factors of the extrudates

Anti-nutritional factors (ANFs) as defined in the introduction of this chapter, are substances which after ingestion, impair digestion. Considering the immediate application of the extrudates as ready to eat snacks food, some of the ANFs were analysed by an external laboratory due to some safety measures and expertise required in the analytical procedures.

The data in Table 7-4 shows a total (97%) deactivation of trypsin inhibitor by extrusion cooking as noted by many scholars (Alonso et al., 2000a, Anton et al., 2009). Trypsin inhibitors are among protease inhibitors which

obstructs the action of protein digestive enzymes in the human system. With the reduction of trypsin inhibitor through extrusion cooking, all available protein could be easily digested. The deactivation of this protease inhibitor may have contributed to the increase in the protein digestibility.

**Table 7-4:** External laboratory results of anti-nutritional factors of native pigeon pea (NPP) and extrudates from treated and non-treated feed material.

| Samples | Cyanide | Phytic acid (g/100g) | Oxalic acid (g/100g)) | Trypsin inhibitor (TIU/g) |
|---|---|---|---|---|
| NPP | ND | 0.72 | 0.01 | 16841 |
| NPPE | ND | 0.71 | 0.01 | 688 |
| HTTE | ND | 0.63 | 0.01 | 224 |
| BBE | ND | 0.64 | 0.01 | 593 |

Values represents the average of replicate analysis, ND: Not detected.

Previous analysis of the native milled powder confirmed the absence of hydrogen cyanide in the pigeon pea thus increasing the safety in the processing and consumption of the extrudate. Similar to cyanide, minute levels of oxalic were detected in both the non-extruded and extruded pigeon pea. The evaluation of the phytic acid remaining after extrusion cooking shows some reduction. Due to the indigestibility of phytic acid in human (attributed to the absent of phytase enzyme) and unavailability of certain minerals (calcium and magnesium) due to the chelation by phytic acid, elimination or reduction to safe level was required. The treatment offered to the feed and in combination with extrusion seems to be more effective in the reduction of phytic acid compared to extrusion of NPPE from non-treated feed. The X-ray diffractogram (**Figure 6-13**) also confirms the reduction of peak at 34º theta (2θ) which was suspected to be from phytin globoid crystal. In contrast to the low reduction obtained in the present thermomechanical extrusion, higher reduction through extrusion has been reported (Alonso et al., 2000a, Anton et al., 2009). Although the level of phytic acids in the extrudates were comparable to cereals such as oats, however, care is required to ensure other nutrients are available in the

extrudate so that any negative impact due to the phytic acid does not affect the nutritional values.

## 7.5 Chapter conclusion

The thermomechanical extrusion contributed to changes in the nutritional composition especially in the digestibility of protein and starch. Having established in chapter 6 that the stable extrudates (NPPE, HTTE and BBE) are ready to eat snacks, the evaluated amino acid could meet the recommended daily intake of some of the essential amino acid if consumption levels were monitored. As recommended, reduction of some anti-nutritional factors through extrusion cooking was achieved especially the near total deactivation (97%) of trypsin inhibitors within the BBE and HTTE. Therefore, based on the nutritional assessment, the stable extrudates produced from low moisture treated feeds could serve as an alternative to improve the food and nutrition security of developing nations especially Nigeria.

# 8 General conclusion

This chapter summarises the major findings as previously discussed in the different chapters as they impact on the degradation of the seed components, disruption of the structural matrix and the formation of expanded snack products. From an overview of literature reports, it was discovered that the non-permeability of seed coat and cotyledon cell walls was thought to contribute to the delay in the hydration and cooking of pigeon pea seed. Similar to other legumes, this delay in hydration and cooking of the seed were generally termed "Hard to cook" by many researchers. Many authors had applied several techniques on the native seed, such as dehulling, overnight (12 hr) soaking at room temperature and boiling with or without salt to reduce this limitation during the conventional utilisation of pigeon pea. Some of these treatments were investigated in the course of this research and their impact discussed.

## 8.1 Physicochemical characteristics of pigeon pea

From the initial experimental analysis in chapter 4 to characterise one of the varieties' (white Agatu) physical and hydration properties, seed material was found to be dense (specific density of 1.2g/ml). The high density is an indication that the internal structure of the seed does not have many voids and hydration into the internal structure (cotyledon) may be impaired. During the preliminary hydration of the same white Agatu seed at 50 °C, a visible change appeared on the seed coat within 30 minutes of hydration. As the change becomes obvious with the seed coat becoming wrinkled, it was very easy to be detached from the cotyledon. Considering that the extent of cooking of seed would depend on the hydration of the seed structural components (seed coat and cotyledon), the seed coat had a two-fold increase in weight during the hydration while the cotyledon only increased by 29%. With this hydration pattern, it could suggest that more

water is absorbed by the seed coat at the start of hydration rather than the cotyledon. Probably, pigeon pea seed coat has a different architecture from the report on many legumes in which the seed coat impairs hydration. Subsequent hydration with the other two varieties (red Agatu and Bayelsa) confirms that the seed have the capacity to absorb water at a level equivalent (100%) to a two-fold increase of their dry weight. Although white Agatu recorded the highest seed weight but the highest hydration capacity was attained by Bayelsa even when the two parameters were supposed to be complementary.

From scientific literature, it was suggested that high levels of polyphenol would decrease hydration rates of the seed coat, but there was no indication that the red Agatu differed from the white Agatu during the current study. It is possible that all the varieties of pigeon pea were stored in optimum condition and the major influence of the hard to cook phenomenon never occurred. However, the current study shows that the pigeon pea is commercially available from Nigeria and that it can be made into ready to eat snacks if the processing conditions are controlled. Further work on correct storage of the pigeon pea could be worthwhile.

Evidence from chemical compositions in Table 4-1show differences due to the different methods (12 hour soaking at room temperature, dehulling and drying) used in preparation of the seed flour. The dehulling of the seed resulted in an increase in starch content (from 35 % in whole seed to 49.96% in dehulled sample). This was attributed to the increase in cotyledon concentration. The effect of soaking caused an overall decrease in the protein concentration as a result of the leaching of soluble proteins during the 12 hour soaking. In spite of the protein loss during soaking period, approximately 20% protein was obtained in all the samples and therefore these could be used to produce a high protein snack. Similar to starch, the soaking and dehulling increased the lipid content though the value (1.65%) was low and adequate for the production of healthy snacks.

A comparison of the native seed flour with wheat flour and maize starch, identified pigeon pea with no breakdown in viscosity. This viscosity profile was an indication that pigeon pea powdered material when hydrated can swell under heat. However, compared to cereals, differences occurred in the values of pasting viscosities of pigeon pea. This may be attributed to the low starch level (35%) of pigeon pea, limited availability of water to starch during gelatinisation, presence of non-starch components or the poor swelling capacity, which is peculiar to legumes. To assist in the confirmation of thermo-mechanical stability of pigeon pea starch, a further better comparison would be necessary. The pasting analysis will be good if conducted with the same starch concentration rather than the 12.5% solid content which may not reflect the influence of the other materials. The moderate concentration used in the RVA pasting profiles may not reflect the behaviour at high solids levels used in snacks food production.

The pasting properties of the three varieties were considered important to select the variety that can be easily utilised in the manufacture of ready to eat snacks. The changes in the behaviour of the native starch granules and other particles of the materials (fibre and protein) in relation to the gelatinisation and swelling of the native granule were recorded as pasting viscosities. The schematic diagram in Figure 8-1was proposed to explain the hydration and swelling behaviour of the native powdered pigeon pea during the pasting. It further describes the measurement of the peak viscosity, the subsequent breakdown of the fragile swollen granule and possible reordering of the materials as detected from the pasting curve in Figure **4-4** to Figure 4-4-6.

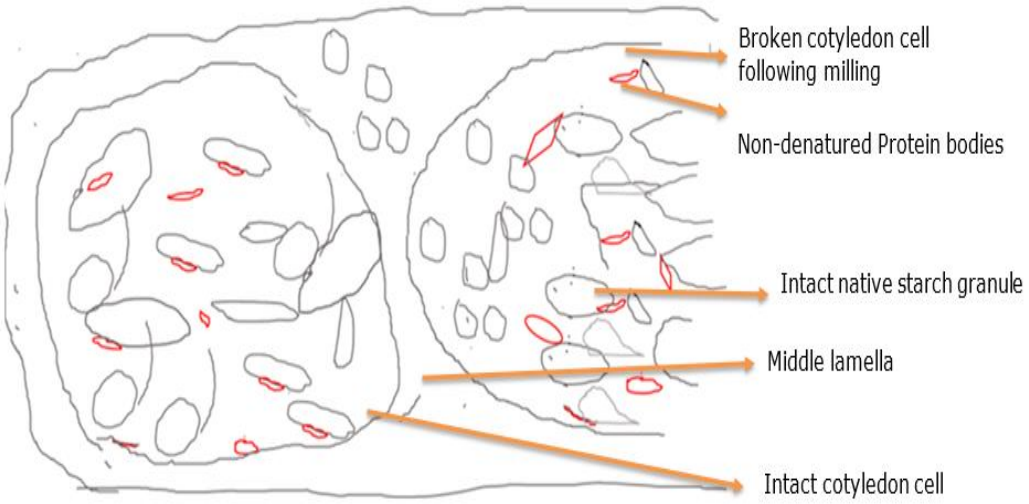



**Figure 8-1:**Schematic diagram showing the hydration of starting pigeon pea powder in cold water at the start of the RVA pasting process.

Some of the starch granule are intact and some were broken along with the cotyledon during milling. Protein in red, starch granule in gray.

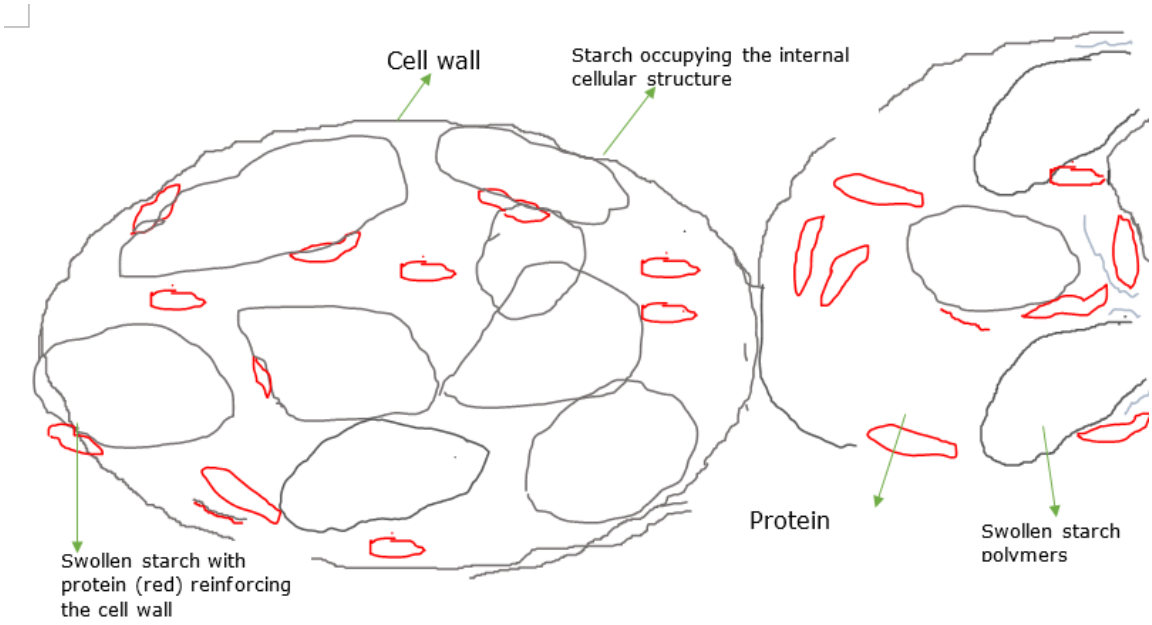



**Figure 8-2:** The schematic diagram of powered pigeon pea at the middle of pasting where swelling is at maximum stage.

Here hydration is very high and some of the swollen materials' components (starch, protein and fibre) are occupying the hydrodynamic volume. Protein: red, starch granule: Oval grey shape.


**Figure 8-3**: Schematic diagram showing the last stage of pasting.

Hydration is slow and the swollen material remnants are in aqueous phase with fragmented cells. Protein: red, starch granule: Oval grey shape

All the three varieties showed similar pasting patterns with limited peak viscosity, though differences due to the varieties, flour preparation methods and presence of non-starch components did affect the actual pasting profiles. Dehulling increased the viscosities presumably due to increase in cotyledon concentration, and therefore the level of starch. The prior hydration, plasticisation and annealing that resulted from the 12-hour conventional soaking and drying at 50 °C may have impacted the pasting behaviour. The peak viscosity displayed is proposed to relate to the hydrodynamic volume of the different seed components (starch, protein and fibre) and their interactions (Figure 8-1). This was later confirmed with the increased peak viscosity of 70 °C HTT and wet milled (Table 5-2). There was no clear impact of hydration followed by drying on pasting profile. It could therefore be concluded that conventional soaking caused little permanent changes to the cellular components that impacted on subsequent hydrodynamic volumes.

Although Bayelsa varieties showed higher paste viscosities, there was little to recommend one variety of seed over another. From the Nigerian consumers (South-Eastern consumers) perspective, the use of Bayelsa variety for snack manufacturing would be expensive due to the high cost of the material. The consumers and farmers would prefer white Agatu due to the low cost of production and adequate availability. It was therefore this white Agatu type of pigeon pea which was studied further. However, the most important things before the conventional processing of pigeon pea for consumption is to improve the hydration of the seed so that more water will be available for starch gelatinisation and deactivation of anti-nutritional factors.

## 8.2 Treatment to improve hydration and seed softening

Based on food safety associated with the traditional 12 hours overnight soaking of the seed, new hydration regimes were investigated. The improvement of the water uptake into the seed was investigated. The hydration of the seed at temperatures (37°C to 90 °C) higher than the room temperature, but lower than endset gelatinisation temperature of pigeon pea starch, improved the water uptake into the seed. Although the hydration technique known as hydrothermal treatment (HTT) was performed in a quiescent environment of not stirring the water bath, the water uptake curve in Figure 5-1,revealed three stages linked to changes in the physical state of the seeds.

The first stage includes a short period (30 min) of rapid water uptake, presumably through diffusion actions where the water uptake was increased by 20%. The native seeds are dense due to the compact internal structure of the cotyledon cells in which starch is embedded in the protein matrix and this is surrounded by cell wall material. There are a few channels between the cells while the cotyledon is then surrounded by the seed coat. Successfully, in this 1$^{st}$ stage of hydration, the seed coat becomes easily

detachable from the cotyledon. This evidence confirmed that the seed coat of pigeon pea is highly permeable and would not interfere with the hydration of the inner structure (cotyledon) where most of the seed components were found. Therefore, the difficulties in the removal of seed coat from the cotyledon and seed coat action to reduce hydration may occur in poorly stored samples giving rise to the hard to cook phenomenon.
In the second hydration stage, the seed obtained its maximum moisture content corresponding to an equilibrium in the water uptake curve. The hydration amount was the same for all temperatures. The temperature dependence of the time to reach the equilibrium moisture content indicated change in the water uptake.

The last stage of the hydration was easily noticed from the water uptake curve in Figure 5-1.This stage showed neither change in the moisture content of the seed nor in physical state of the seed.

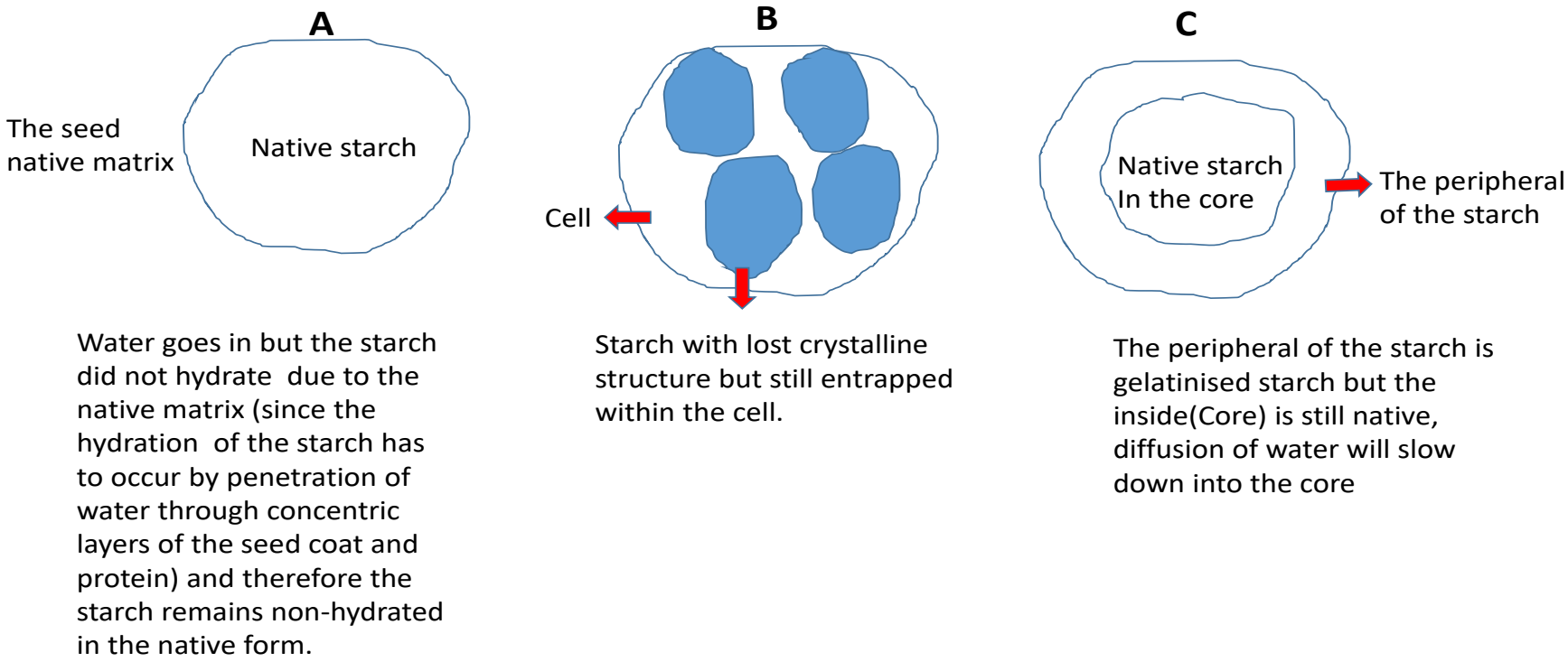



**Figure 8-4:** The different scenario limiting the hydration of starch during hydrothermal treatment of the seed.

Since at higher temperature, the maximum hydration of the seed could occur less than 75% of the conventional soaking time, this method of hydration could scale-up the processing of pigeon pea. In addition, Figure 8-4 also shows how the seed starch may be affected at different scenario during the hydration at the hydrothermal treatment of 37-90 °C.
Importantly, in Figure 8 4, the changes which impacted on the starch granule during HTT affects overall assessment of the HTT seed. Evidence from the force deformation chart in Figure 5 4 showed that less force was required to puncture the seeds hydrated at the highest temperature (90 °C) even when the equilibrium moisture content was the same as for sample hydrated at lower temperatures. Also this method used to measure the hydrated seed hardness (maximum deformation force) was considered to indicate cell-cell adhesion. The internal moisture is relevant to this hardness measurement, but gelatinisation of the starch also plays a role in the texture, causing the loss of native starch structure. While the DSC thermal properties in Table 5-1 confirmed the absence of any endotherm for seeds hydrated at 90 °C, samples hydrated at 37 °C -70 °C, shows little change from the native properties. With these changes in the structures and components of the seed at 90 °C, it can be confirmed that starch gelatinisation which resulted in pigeon pea seed softening occurred when sufficient moisture and appropriate temperature were available during the cooking.

Mobility can occur through temperature, but also the plasticisation levels are critical as seen in state diagram (Figure 2-8). The poor swelling of untreated samples demonstrate that the loss of starch crystallites is necessary for the material to swell. However, the hydrothermal treatments at 90 °C for 2-4 hours shows that both temperature and moisture were necessary for the starch gelatinisation transition to occur. But when treatments below this temperature was used, no permanent starch

transition occurred. Possibly, preliminary process may not be relevant for the production of high quality snacks foods as the cooling of the cooked material may cause retrogradation of the starch and hence high energy may be later required for melting of retrograded starch before processing of the snacks. However, the application of high energy inputs in the 1st and 2ndprocessing stages to cook pigeon pea could not be recommended.

To have the cell to cell wall breakdown, high hydration rates but limited energy inputs, chemical intervention targeting the cell walls were investigated. These techniques include a short time hydrothermal steam treatment at 90 °C for 5 mins with or without alkali and a cell wall degradation treatment. It was expected that the replacement of the quiescence hydration in a water bath to more stirring hydration (mild shear hydration) in steam vessel would improve the seed softening.
Macromolecules cannot undergo transition unless they have the necessary mobility. But when the native milled powders were heated without any stirring they swelled to a greater extent than the native seed sample. This shows that as water penetrates the powders and cause hydration of the materials, the starch could gelatinise, but the swelling must be limited as the values for the swelling capacity are low. But when the same native milled material was pasted under limited shears, the values for the treated samples peak viscosity were greater than the native samples, presumably showing that the cellular confines in the treated were less robust. However, the pasting profile shows that the breakdown of the native swollen matrices is not great on pasting, demonstrating that the samples may still be poorly expanded and that the structures are robust against shear. All the samples subjected to thermal, alkaline and enzymatic treatments showed no measurable cold water viscosity in addition to retention of gelatinisation enthalpy values equivalent to those in the native pigeon pea.

The treated milled samples could be expected to behave in the following ways during second heating process when added to excess water with or without shear;

1. If there has been no breakdown of the macromolecule structure then little hydration or swelling is expected (as occurred in sample A1 (milled native sample), even with shear
2. If all the macromolecular structures were broken down, the samples could be expected to dissolve, but this may not occur for some samples even with shear.
3. If the starch has lost its native structure, but is confined within a matrix, then its ability to swell may be reduced.
4. If particles are large (for example the seeds) then hydration at the surface of the materials may allow the starch to gelatinise and swell, but in doing so this could restrict water to the internal portion of the particulate and reduce the ability of the internal starch to hydrate and loose crystallinity.

At this stage after the cell wall degradation treatment, the samples studied in this thesis can be considered as representing the following:

1. Whole pigeon pea seeds; where there is a seed coat or not and the cellular structures of the cotyledons remains in tact
2. Milled native pigeon pea seed; milling may break cell walls and expose the internal cell materials including the native starch and the matrix and storage proteins.
3. Treated Pigeon pea seed: the hydration with different materials was designed to breakdown the cellular walls within the cotyledon.
4. Milled treated pigeon pea seed; milling may further break cell walls and expose the internal cell materials including the native starch and the matrix and storage proteins.

The two major factors that were considered for the behaviour of the pigeon pea were the loss of starch crystallinity as an index of cooking of the seed and the ability of the comminuted samples to swell showing that hydrating materials are not confined within a non-expandable network.
Other factors which influences the processing behaviour of pigeon pea includes; the intact cell wall, or cell walls reinforced with other materials, such as amylose or protein or constrictions to starch swelling due to materials at the surface of the starch (This is shown in the Figure 8-1).

## 8.3 Extrusion

Since the cell wall degradation treatment was unable to effect sufficient changes in the starch crystalline structure, a severe shearing treatment such as thermos mechanical extrusion, known for causing severe disruption of structures including reducing starch crystalline order, was applied. The equipment used was a pilot scale twin screw extruder fitted with two screws. These screws had severe mixing elements and reverse screws to maximise breakdown of the materials. It is regrettable that the calculation for the specific mechanical energy as calculated according to the manufacturers is not likely to be correct and is an over estimate, perhaps doubling the values reported in the literature for equivalent processes. The torque values do show that significant shear was added to the samples when extruded at low (<12%) moisture.
When the non-treated milled pigeon pea materials were extruded at moisture contents of 22% dwb in the barrel of the extruder and a die temperature of 150 °C, expansion did not occur, but a homogenous cohesive string of material was formed. In some samples there was clear evidence of particulates in the melted mass of material, suggesting that some of the particles had not melted. Further analysis to determine the extent of starch conversion in this extrudate confirmed that the majority of

the starch granules were still in the native state though high thermal input rather than mechanical forces increases the starch conversion. This was explained by suggesting that slippage of the viscous mixtures reduces the material interactions within the barrel and low mechanical forces will be generated leading to low starch conversion. Several factors may combine and be responsible for the low starch conversion of the high moisture feed during extrusion. The poor hydration characteristics of the milled pigeon pea material and the short residence time in the extruder, may mean that the feed particles did not hydrate. The non-absorbed water present could then lubricate and form a water layer between the poorly hydrated particles and hence less frictional force would be developed. Though a product was formed form high moisture extrusion but because of the low starch conversion, the product would require additional processing to make it expand to form an edible snack.

A reduction of the preliminary extrusion feed moisture and an increase in feed rate increased the torque and expanded products were obtained. Extrusion with the low moisture feed (11%) from the cell wall degradation (hydrothermal and alkaline) treated seed gave the extrudate with the most positive product characteristics. This was attributed to the degradation of cell wall and disruption of native matrix earlier established in section 5.3. All products assessments (cold-water viscosity, DSC gelatinisation enthalpy and X-ray) confirmed higher starch conversion. Extrudate BBE from low moisture alkaline treated feed material manifested the highest product qualities compared to all other samples.

The evaluation of the extrudates microstructure with Scanning Electron Microscope (SEM) confirmed complete disruption of native cellular structure. The assemblies of new cellular structure filled with air spaces were visible in extrudates developed from low moisture feed and high feed rate. Although HTTHME had the highest cold-water viscosity but seemed to demonstrate a unique image of coalesced cells. No traces of native starch

granule were visible in the images of the low moisture extrusion. Hence the native granules must have been disrupted or degraded during the thermomechanical extrusion. With the microstructural images plus the results of all the assessments that indicated high starch conversion in low moisture extrudates it was confirmed that high levels of starch de-structuring were necessary to achieve an expanded product.

Further observation with epifluorescence microscope revealed the effect of the interactions of the seed chemical components in the extrudate cellular formations. Due to the limited starch conversion in NPPHME, the microstructure became visible as a complex mass with the mixed disrupted cellular components enclosed within the cell compartment. No sign of formation of air cellular units could be detected. In the BBE product, which had the highest product attributes, no segregation of the chemical components was visible except a limited portion of protein separation towards the edge of the cells. The signs of air cellular units became obvious in BBE. It is suggested that for good expansion, not only should the starch be degraded from its native state, but all the components should become homogenous.

The improvement in extrudate characteristics following the extrusion of low moisture cell wall degradation treated feed has shown that combined cell wall degradation treatment and thermomechanical extrusion were adequate in the disruption of pigeon pea native cellular matrix and formation of stable products. Sufficient hydration and shear are necessary to break down native structures (starch and protein) and stop compartmentalisation of the materials so that a homogenous melt can be formed. It is this molten material that allows an elastic bubble wall to be formed and allows water vapour expansion to create an expanded crispy product which is the base for a ready to eat snack. Since the essence of hydration and thermal treatment was to render the material nutritional and

safe to eat. Following the extrusion cooking of the combined hydrothermal and alkaline treatments feed, the reduction of the native anti-nutritional factors ensured an increase in the digestibility and availability of nutrients from pigeon pea.

In conclusion, this current study has shown that pigeon pea which is commercially available in Nigeria can be made into ready to eat expanded snacks if adequate processing conditions were applied.

## 8.4 Recommendations for further studies

Having monitored and evaluated the transition of pigeon pea seed from different processing regimes, it is likely that many factors other than the ones investigated or explored during this research may have influenced the processing characteristics of pigeon pea. Although stable extrudates, which are safe, and formed ready to eat snacks were created in this study certain aspects still would need further investigations to optimise the extrusion cooking. Based on some considerations, the following suggestions are being recommended for further studies;

### 8.4.1 Starting material (Native pigeon pea seed)

1. The hard to cook factor has been suggested to be related to post harvest factors. May be due to the storage condition of 20 °C, no differences were observed in the physicochemical characteristics of the different batches of 5 months' post harvested white Agatu pigeon pea used throughout this study. Therefore, further work may be required to better understand the relevance of pigeon pea storage to the processing behaviour of the seed.
2. To improve the extrudate qualities, newly harvested pigeon pea seed may be preferable. Extrusion being a quick and easy continuous process

could be an ideal mechanism for processing the fresh harvest. This could be investigated.

### 8.4.2 First stage processing (hydration and seed softening)

1. If hydrothermal treatments are required then a jacketed system with enhanced drainage that will easy allow the draining of water and straining of the seed after hydrothermal treatment could be necessary. This will reduce excess imbibition of water into the seed which would require more cost to dry off. The mass energy balance for the whole of the process should be evaluated.
2. A feature of the processes is that the pigeon pea material as well as being difficult to hydrate is also difficult to mill. A more efficient mill capable of producing a homogenous ground powder with different particle sizes could be investigated so optimum levels can be established.
3. Further work on evaluating different levels of the bicarbonate salts and impact of other food grade alkaline solutions on the cell wall degradations may improve the feed quality for extrusion cooking.

### 8.4.3 Extrusion

1. Different levels of shear using modified screws could enable even more shear to be introduced during processing. This may allow even better extruded products to be formed.
2. Pigeon pea could be mixed with another starch to encourage more expansion and this could be investigated.

### 8.4.4 Extrudate quality

1. At this time the acceptability of the extrudates has not been assessed as a ready to eat snack. Some sensory evaluation and consumer research

is necessary to see if this pigeon pea based product could be successful in the market.

2. Future extrusion of feed with more solubilised cell wall materials and degraded starch may have a role in nutrition when high energy levels foods are required. For example, for children's products in order to reduce any interference with their digestive system. The converse is that leaving the structures in place may allow a snack product with a low glycemic index. The correct route would need some input from nutritionists and policy makers.

3. The shelf life of the stable extrudates and their packaging would require to be established through monitoring product characteristics before it was commercialised.

4. Most ready to eat snacks are flavoured and this has not yet been considered.

### 8.4.5 Future work and dissemination of knowledge from this study

1. This work described in this thesis has underlined the relevance of cell walls and other materials on the functioning of the hydration process and the subsequent behaviour of the starches. This is not a common way to think about the action of processing on starchy materials. It is recommended that processing of other starchy materials is reviewed in terms of the other components that could compartmentalise the starch.

2. Application of advanced analytical methods such as use of NMR, FTIR and microscopy could help with the understanding when
investigating and monitoring other structural changes in pigeon pea microstructures during processing.

3. There is currently an upsurge in those interested in processing legumes to make snacks. The work from this thesis should be disseminated so learnings can assist others in the manufacture of other legume products.

### 8.4.6 Summary of research findings

Reports have shown that the hard to cook textural defect of pigeon pea was initiated through storage at high humidity and presence of seed coat and cell wall. In contrast to the above report, it was observed during the study of hydration pattern of pigeon pea that the seed coat becomes wrinkled and easily detached from the cotyledon within 30 minutes of soaking in water. Hence, the seed coat did not show to have pose any difficult in the hydration of the seed. In addition, the hydration pattern shows that the seed is a well hydrating seed, a characteristic confirmed by the rapid increase in the water uptake from the initial moisture content of the seed and the absence of lag phase at the start of hydration.

Some conventional processing methods such as soaking and dehulling shows some significant differences at $P<0.05$ on the physicochemical properties. Dehulling increased the chemical composition due to the increase in cotyledon concentration while the 12-hour conventional soaking resulted in the reduction of the protein content. Due to the non-significant of the varieties in the physicochemical properties, white Agatu was selected since it is the most available and easily cultivated. However, the hydration at higher temperature of 70 °C and 90 °C shorten the time required to obtain the equilibrium moisture content. Relating, the differences in the reduction of seed hardness to thermal properties shows that reduction of hydrated seed hardness seed at below (37-70 °C) the pigeon pea starch gelatinisation temperature is due to cell to cell separation from water penetration into the cellular interstitial spaces which causes plasticisation and dilution effect. However large reduction of hydrated seed hardness at 90 °C occurred due to the softening of the seed after starch gelatinisation. Therefore, softening of the seed structural matrix below starch

gelatinisation temperature does not suggest that the seed has been cooked except the loss of the starch crystallinity.

Milling (wet or dry) and drying after hydration had an impact in the materials swelling behaviour at quiescent and non-quiescent conditions. The swelling depends on the exposure of the starch granule from the encapsulated matrix and the hydrodynamic volume occupied by the seed chemical components. Considering that higher moisture content was attained when hydrating the seed at a longer time (60-90 min) which is not cost effective removing the excess water prior to extrusion, the impact of a short time (5 min) hydrothermal treatment at 90 °C with or without alkaline solution and enzyme were applied to degrade the cell wall material since it contributes to the delay in hydration of the seed. Although the treatment impacted the degradation of the cell wall material, but the starch native structure remained unaltered. Probably due to the short treatment time, water was not sufficient to hydrate the starch so that gelatinisation can occur.

Since the native starch structure was difficult to disrupt during the short time cell wall degradation treatment, a more severe treatment known for tearing the granule apart was applied in the form of thermomechanical extrusion. With this method, a ready to eat expanded products with good product attribute and with high starch conversion (high disruption of starch crystalline structure) were obtained at the extrusion conditions of 14 kg/h feed rate, 150 °C die temperature and 11 % feed moisture. At this condition, the slippage caused by high moisture feed was reduced and through the formation of homogenous viscoelastic melt, higher expansion between 3-4-fold die diameters was obtained from the treated feed. As confirmed by the fluorescence image of the extrudates, the product quality is enhanced by the compatibility of all the materials components (starch,

protein and fibre). Although, the X-ray diffraction pattern of native pigeon pea is typical of C-type starch polymorphs, but a diffraction peak at 34º theta (2θ) was obtained (proposed to be globoid crystals of phytic acid salt; an anti-nutritional factor) which has not been reported of other legumes though reduced after extrusion cooking. The elimination of this peak in the X-ray diffraction peak of the isolated starch from pigeon pea, confirmed that it is crystallites from non-starch components possibly globoid crystals of phytic acid (anti-nutritional factor) with complexed mineral salt.

Based on the characterisation of the pigeon pea as a processing material and with the formation of expanded ready to eat snack product, this research could be a guide for material characterisation and commercial production of extruded products from pigeon pea and similar legume seeds.

# References

ACEVEDO, B. A., AVANZA, M. V., CHÁVES, M. G. & RONDA, F. 2013. Gelation, thermal and pasting properties of pigeon pea (Cajanus cajan L.), dolichos bean (Dolichos lablab L.) and jack bean (Canavalia ensiformis) flours. *Journal of Food Engineering,* 119**,** 65-71.

ADEBOWALE, K., HENLE, T., SCHWARZENBOLZ, U. & DOERT, T. 2009. Modification and properties of African yam bean (Sphenostylis stenocarpa Hochst. Ex A. Rich.) Harms starch I: Heat moisture treatments and annealing. *Food Hydrocolloids,* 23**,** 1947-1957.

AGUILERA, J. & RIVERA, R. 1992. Hard-to-cook defect in black beans: hardening rates, water imbibition and multiple mechanism hypothesis. *Food Research International,* 25**,** 101-108.

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

AGUILERA, J. M., STANLEY, D. W. & BAKER, K. W. 2000. New dimensions in microstructure of food products. *Trends in Food Science & Technology,* 11**,** 3-9.

AGUNBIADE, S. O. 1996. Cookability of African yambean, Sphenostyrrs Srenocrarp (Hochst ex. A. Rich) Harma. *Nahrung 40 (1996) Nr. 1, S. 37-40 0,* Nahrung 40 (1996) Nr. 1, S. 37-40 0**,** Nahrung 40 (1996) Nr. 1, S. 37-40 0.

AGUNBIADE, S. O. & LONGE, O. G. 1999. The physico-functional characteristics of starches from cowpea (Vigna unguiculata), pigeon pea (Cajanus cajan) and yambean (Sphenostylis stenocarpa). *Food Chemistry,* 65**,** 469-474.

AKDOGAN, H. 1996. Pressure, torque, and energy responses of a twin screw extruder at high moisture contents. *Food Research International,* 29**,** 423-429.

AKINJAYEJU, O. A. & AJAYI, O. 2011. Effects of Dehulling on Functional and Sensory Properties of Flours From Black Beans (Phaseolus Vulgaris). *Food and Nutrition Sciences,* 2**,** 344-349.

ALBERSHEIM, P., DARVILL, A., ROBERTS, K., SEDEROFF, R. & STAEHELIN, A. 2011. *Plant Cell Walls from Chemistry to Biology,* New York, Garland Science.

ALCÁZAR-ALAY, S. C. & MEIRELES, M. A. A. 2015. Physicochemical properties, modifications and applications of starches from different botanical sources. *Food Science and Technology (Campinas),* 35**,** 215-236.

ALONSO, R., AGUIRRE, A. & MARZO, F. 2000a. Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food chemistry,* 68**,** 159-165.

ALONSO, R., ORÚE, E., ZABALZA, M. J., GRANT, G. & MARZO, F. 2000b. Effect of extrusion cooking on structure and functional properties of pea and kidney bean proteins. *Journal of the Science of Food and Agriculture,* 80**,** 397-403.

AMBIGAIPALAN, P., HOOVER, R., DONNER, E. & LIU, Q. 2014. Starch chain interactions within the amorphous and crystalline domains of pulse starches during heat-moisture treatment at different temperatures and their impact on physicochemical properties. *Food chemistry,* 143**,** 175-184.

ANTON, A. A., FULCHER, R. G. & ARNTFIELD, S. D. 2009. Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (Phaseolus vulgaris L.) flour: Effects of bean addition and extrusion cooking. *Food Chemistry,* 113**,** 989-996.

ANUONYE, J. 2012. Some functional properties of extruded acha/soybean blends using response surface analysis. *African Journal of Food Science,* 6**,** 269-279.

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

APATA, D. F. 2008. Effect of cooking methods on available and unavailable carbohydrates of some tropical grain legumes. *African Journal of Biotechnology,* 7.

ARNOLDI, A., ZANONI, C., LAMMI, C. & BOSCHIN, G. 2015. The role of grain legumes in the prevention of hypercholesterolemia and hypertension. *Critical Reviews in Plant Sciences,* 34**,** 144-168.

AYKROYD, W. R., DOUGHTY, J. & WALKER, A. F. 1982. *Legumes in Human Nutrition*, Food and Agriculture Organization of the United Nations.

BAKER, A. A., MILES, M. J. & HELBERT, W. 2001. Internal structure of the starch granule revealed by AFM. *Carbohydrate research,* 330**,** 249-256.

BALL, S., GUAN, H.-P., JAMES, M., MYERS, A., KEELING, P., MOUILLE, G., BULÉON, A., COLONNA, P. & PREISS, J. 1996. From glycogen to amylopectin: a model for the biogenesis of the plant starch granule. *Cell,* 86**,** 349-352.

BELITZ, H.-D., GROSCH, W. & SCHIEBERLE, P. 2009. Legumes. *Food Chemistry***,** 746-769.

BERRIOS, J. D. J., WOOD, D. F., WHITEHAND, L. & PAN, J. 2004. SODIUM BICARBONATE AND THE MICROSTRUCTURE, EXPANSION AND COLOR OF EXTRUDED BLACK BEANS. *Journal of Food Processing and Preservation,* 28**,** 321-335.

BEWLEY, J. D. & BLACK, M. 1994. *Seeds: Physiology of Development and Germination*, Springer US.

BNF, B. N. F. 2017. *Dietary Fibre* [Online]. Available: https://www.nutrition.org.uk/nutritionscience/nutrients-food-and-ingredients/dietary-fibre.html [Accessed].

BOGRACHEVA, T. Y., MORRIS, V., RING, S. & HEDLEY, C. 1998. The granular structure of C-type pea starch and its role in gelatinization. *Biopolymers,* 45**,** 323-332.

BOHN, L., MEYER, A. S. & RASMUSSEN, S. K. 2008. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Journal of Zhejiang University Science B,* 9**,** 165-191.

BOYE, J., MA, C. & HARWALKAR, V. 1997. Thermal denaturation and coagulation of proteins. *Food Protein and Their Applications.* NEW YORK-: CRC Press.

BRENNAN, C. S., SUTER, M., LUETHI, T., MATIA-MERINO, L. & QVORTRUP, J. 2008. The relationship between wheat flour and starch pasting properties and starch hydrolysis: effect of non-starch polysaccharides in a starch gel system. *Starch-Stärke,* 60**,** 23-33.

BRENNAN, M. A., DERBYSHIRE, E., TIWARI, B. K. & BRENNAN, C. S. 2013. Ready-to-eat snack products: the role of extrusion technology in developing consumer acceptable and nutritious snacks. *International Journal of Food Science & Technology,* 48**,** 893-902.

BRUMMELL, D. A. 2006. Cell wall disassembly in ripening fruit. *Functional Plant Biology,* 33**,** 103-119.

BULÉON, A., COLONNA, P., PLANCHOT, V. & BALL, S. 1998. Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules,* 23**,** 85-112.

BUTT, M. S., TAHIR-NADEEM, M., AHMAD, Z. & SULTAN, M. T. 2008. Xylanases and their applications in baking industry. *Food Technology and Biotechnology,* 46**,** 22-31.

Chakraborty, S. R. , Singh, D .S .,Kumbhar, B. K., Chakraborty, S 2011. Process optimisation with respect to the expansion ratios of millet and legume (Pigeon pea) based extruded snacks. Journal of Food Process Engineering, 34, 777-791.

CAMIRE, M. E. 2001. Extrusion and Nutritional quality *In:* GUY, R. (ed.) *Extrusion cooking: technologies and applications.* Woodhead publishing.

CHANVRIER, H., DESBOIS, F., PEROTTI, F., SALZMANN, C., CHASSAGNE, S., GUMY, J.-C. & BLANK, I. 2013. Starch-based extruded cereals enriched in fibers: a behavior of composite solid foams. *Carbohydrate polymers,* 98**,** 842-853.

CHAPARRO ACUÑA, S. P., GIL GONZÁLEZ, J. H. & ARISTIZÁBAL TORRES, I. D. 2012. Physicochemical characteristics and functional properties of vitabosa (mucuna deeringiana) and soybean (glycine max). *Food Science and Technology (Campinas),* 32**,** 98-105.

CHUNG, H.-J., LIU, Q., HOOVER, R., WARKENTIN, T. D. & VANDENBERG, B. 2008. In vitro starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars. *Food chemistry,* 111**,** 316-321.

COURTIN, C. M. & DELCOUR, J. A. 2002. Arabinoxylans and Endoxylanases in Wheat Flour Bread-making. *Journal of Cereal Science,* 35**,** 225-243.

COZZONE, A. J. 2010. Proteins: Fundamental chemical properties. *eLS*.

CUNIN, C., HANDSCHIN, S., WALTHER, P. & ESCHER, F. 1995. Structural changes of starch during cooking of durum wheat pasta. *LWT - Food Science and Technology,* 28**,** 323-328.

DAMODARAN, S. 2007. Amino acids, peptides and proteins. *In:* DAMODARAN, S., PARKIN, K. L. & FENNEMA, O. R. (eds.) *Fennema's food chemistry.* CRC press.

DE VRIES, R. P. & VISSER, J. 2001. Aspergillus enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and molecular biology reviews,* 65**,** 497-522.

DEBEAUJON, I., LÉON-KLOOSTERZIEL, K. M. & KOORNNEEF, M. 2000. Influence of the Testa on Seed Dormancy, Germination, and Longevity in Arabidopsis. *Plant Physiology,* 122**,** 403-414.

DEL VALLE, J., COTTRELL, T., JACKMAN, R. & STANLEY, D. 1992a. Hard-to-cook defect in black beans: the contribution of proteins to salt soaking effects. *Food Research International,* 25**,** 429-436.

DEL VALLE, J. M., COTTRELL, T. J., JACKMAN, R. L. & STANLEY, D. W. 1992b. Hard-to-cook defect in black beans: the contribution of proteins to salt soaking effects. *Food Research International,* 25**,** 429-436.

DEL VALLE, J. M. & STANLEY, D. W. 1995. Reversible and irreversible components of bean hardening. *Food Research International,* 28**,** 455-463.

DEL VALLE, J. M., STANLEY, D. W. & BOURNE, M. C. 1992c. WATER ABSORPTION and SWELLING IN DRY BEAN SEEDS. *Journal of Food Processing and Preservation,* 16**,** 75-98.

DELLA VALLE, G., TAYEB, J. & MELCION, J. 1987. Relationship of extrusion variables with pressure and temperature during twin screw extrusion cooking of starch. *Journal of Food Engineering,* 6**,** 423-444.

DELLA VALLE, G., VERGNES, B., COLONNA, P. & PATRIA, A. 1997. Relations between rheological properties of molten starches and their expansion behaviour in extrusion. *Journal of Food Engineering,* 31**,** 277-295.

DHITAL, S., BHATTARAI, R. R., GORHAM, J. & GIDLEY, M. J. 2016. Intactness of cell wall structure controls the in vitro digestion of starch in legumes. *Food & function,* 7**,** 1367-1379.

DIAZ, J. V., ANTHON, G. E. & BARRETT, D. M. 2007. Nonenzymatic degradation of citrus pectin and pectate during prolonged heating: effects of pH, temperature, and degree of methyl esterification. *Journal of agricultural and food chemistry,* 55**,** 5131-5136.

DOUBLIER, J., COLONNA, P. & MERCIER, C. 1986. Extrusion cooking and drum drying of wheat starch. II. Rheological characterization of starch pastes. *Cereal Chem,* 63**,** 240-246.

DUHAN, A., KHETARPAUL, N. & BISHNOI, S. 1998. Improvement in starch digestibility (in vitro) of various Pigeonpea cultivars through processing and cooking. *Ecology of Food and Nutrition,* 37**,** 557-568.

DUKE, S. H., KAKEFUDA, G. & HARVEY, T. M. 1983. Differential leakage of intracellular substances from imbibing soybean seeds. *Plant Physiology,* 72**,** 919-924.

E BORGES, E. E. D. L., DA MATA ATAÍDE, G. & MATOS, A. C. B. 2015. Micropilar and embryonic events during hydration of Melanoxylon brauna seeds. *Journal of Seed Science,* 37.

ELLIASON, A. & GUDMUNDSSON, M. 2006. Starch :physicochemical and functional aspects *In:* ELLIASON.A. (ed.) *Carbohydrates in Food.* Boca raton Florida: CRC.

ENGLYST, H. N. & HUDSON, G. J. 1996. The classification and measurement of dietary carbohydrates. *Food Chemistry,* 57**,** 15-21.

ENWERE, N. 1998. Foods of plant origin. Enugu. Nsukka Afroorbis Publications.

ENWERE, N., MCWATTERS, K. & PHILLIPS, R. 1998. Effect of processing on some properties of cowpea (Vigna unguiculata), seed, protein, starch, flour and akara. *International journal of food sciences and nutrition,* 49**,** 365-373.

ENYISI, S. I., UMOH,V.J., WHONG, C.M.Z.,ABDULLAHI,I.O., ALABI,O. 2014. Chemical and nutritional value of maize and maize products obtained from selected markets in Kaduna State, Nigeria

*African Journal of Food Science and Technology,* 5**,** 100-104.

ERTEKIN, M. & KIRDAR, E. 2010. Effects of seed coat colour on seed characteristics of honeylocust (Gleditsia triacanthos). *African Journal of Agricultural Research,* 5**,** 2434-2438.

FASIDI, I. 1981. Influence of chemical and physical properties of pigeon peas on cooking quality. *Die Nahrung,* 25**,** 949-953.

FASOYIRO, S. B., AKANDE, S. R., AROWORA, K. A., SODEKO, O. O., SULAIMAN, P. O., OLAPADE, C. O. & ODIRI, C. E. 2010. Physico-chemical and sensory properties of pigeon pea (Cajanus cajan) flours. *African Journal of Food Science,* 4**,** 120-126.

FASOYIRO, S. B., AROWORA, K. A. & AJANI, A. O. 2017. Storage and Sensory Qualities of Extrudate-Like Maize–Pigeon Pea Snacks Processed by Low-Cost Technology. *Journal of Food Processing and Preservation,* 41**,** n/a-n/a.

FERREIRA, R. E., CHANG, Y. K. & STEEL, C. J. 2011. Influence of wheat bran addition and of thermoplastic extrusion process parameters on physical properties of corn-based expanded extruded snacks/Influencia da adicao do farelo de trigo e de parametros do processo de extrusao termoplastica nas propriedades fisicas de

snacks extrudados expandidos de milho. *Alimentos e Nutricao (Brazilian Journal of Food and Nutrition),* 22**,** 507-521.

FESSEL, S. A., VIEIRA, R. D., CRUZ, M. C. P. D., PAULA, R. C. D. & PANOBIANCO, M. 2006. Electrical conductivity testing of corn seeds as influenced by temperature and period of storage. *Pesquisa Agropecuária Brasileira,* 41**,** 1551-1559.

FORTE, D. 2011. The role of ingredients in extrusion processing. *Process plant network*.

FREDRIKSSON, H., SILVERIO, J., ANDERSSON, R., ELIASSON, A. C. & ÅMAN, P. 1998. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. *Carbohydrate Polymers,* 35**,** 119-134.

GALLANT, D. J., BOUCHET, B. & BALDWIN, P. M. 1997. Microscopy of starch: evidence of a new level of granule organization. *Carbohydrate Polymers,* 32**,** 177-191.

GARCIA-VELA, L., DEL VALLE, J. & STANLEY, D. 1991. Hard-to-cook defect in black beans: the effect of soaking in various aqueous salt solutions. *Canadian Institute of Food Science and Technology Journal,* 24**,** 60-67.

GARCIA-VELA, L. A. & STANLEY, D. W. 1989. Protein Denaturation and Starch Gelatinization in Hard-To-Cook Beans. *Journal of Food Science,* 54**,** 1284-1286.

GARCIA, E. & LAJOLO, F. M. 1994. Starch Alterations in Hard-To-Cook Beans (Phaseolus vulgaris). *Journal of Agricultural and Food Chemistry,* 42**,** 612-615.

GHUMMAN, A., KAUR, A. & SINGH, N. 2016. Impact of germination on flour, protein and starch characteristics of lentil (Lens culinari) and horsegram (Macrotyloma uniflorum L.) lines. *LWT - Food Science and Technology,* 65**,** 137-144.

GIDLEY, M. J., COOKE, D. & WARD-SMITH, S. 1993. Low moisture polysaccaharide system:thermal and spectroscopic aspects. *In:* BLANSHARD, J. M. V. & LILLFORD, P. J. (eds.) *The Glassy state in Foods.* Loughborough: Nottigham University Press.

GIL, J., NADAL, S., LUNA, D., MORENO, M. T. & HARO, A. D. 1996. Variability of Some Physico-chemical Characters in Desi and Kabuli Chickpea Types. *Journal of the Science of Food and Agriculture,* 71**,** 179-184.

GIMBA, D., ABDUL, K. & GANGAS, O. Necessity for sufficient processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds.

GOMEZ, M. & AGUILERA, J. 1984. A physicochemical model for extrusion of corn starch. *Journal of Food Science,* 49**,** 40-43.

GUNARATNE, A. & HOOVER, R. 2002. Effect of heat–moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydrate Polymers,* 49**,** 425-437.

GÜZEL, D. & SAYAR, S. 2012. Effect of cooking methods on selected physicochemical and nutritional properties of barlotto bean, chickpea, faba bean, and white kidney bean. *Journal of Food Science and Technology,* 49**,** 89-95.

HARPER, J. M. 1981. *Extrusion of Foods,* Boca Raton, Florida CRC Press Inc.

HARPER, J. M. 1992. A Comparative Analysis of Single and Twin -Screw Extruders *In:* KOKINI, J. L., HO, C.-T. & KARWE, M. V. (eds.) *Food extrusion science and technology.* New York: M. Dekker.

HASJIM, J., LI, E. & DHITAL, S. 2013. Milling of rice grains: Effects of starch/flour structures on gelatinization and pasting properties. *Carbohydrate polymers,* 92**,** 682-690.

HENTGES, D., WEAVER, C. & NIELSEN, S. 1991. Changes of Selected Physical and Chemical Components in the Development of the Hard-to-Cook Bean Defect. *Journal of Food Science,* 56**,** 436-442.

HINCKS, M. & STANLEY, D. 1986. Multiple mechanisms of bean hardening. *International Journal of Food Science & Technology,* 21**,** 731-750.

HINCKS, M. J. & STANLEY, D. W. 1987. LIGNIFICATION: EVIDENCE FOR A ROLE IN HARD-TO-COOK BEANS. *Journal of Food Biochemistry,* 11**,** 41-58.

HOHLBERG, A. I. & STANLEY, D. W. 1987. Hard-to-cook defect in black beans. Protein and starch considerations. *Journal of Agricultural and Food Chemistry,* 35**,** 571-576.

HOLM, J., LUNDQUIST, I., BJÖRCK, I., ELIASSON, A.-C. & ASP, N.-G. 1988. Degree of starch gelatinization, digestion rate of starch in vitro, and metabolic response in rats. *The American journal of clinical nutrition,* 47**,** 1010-1016.

HOOVER, R. 2001. Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers,* 45**,** 253-267.

HOOVER, R. 2010. The impact of heat-moisture treatment on molecular structures and properties of starches isolated from different botanical sources. *Critical Reviews in Food Science and Nutrition,* 50**,** 835-847.

HOOVER, R. & MANUEL, H. 1996. Effect of heat—moisture treatment on the structure and physicochemical properties of legume starches. *Food Research International,* 29**,** 731-750.

HOOVER, R. & RATNAYAKE, W. S. 2002. Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chemistry,* 78**,** 489-498.

HOOVER, R. & SOSULSKI, F. 1986. Effect of Cross-Linking on Functional Properties of Legume Starches. *Starch - Stärke,* 38**,** 149-155.

HOOVER, R., SWAMIDAS, G. & VASANTHAN, T. 1993. Studies on the physicochemical properties of native, defatted, and heat-moisture treated pigeon pea (Cajanus cajan L) starch. *Carbohydrate Research,* 246**,** 185-203.

HSU, K. H., KIM, C. J. & WILSON, L. A. 1983. Factors Affecting Water UPtake of Soybeans During Soaking. *Cereal Chemistry,* 60**,** 208-211.

HUANG, J., SCHOLS, H. A., VAN SOEST, J. J. G., JIN, Z., SULMANN, E. & VORAGEN, A. G. J. 2007. Physicochemical properties and amylopectin chain profiles of cowpea, chickpea and yellow pea starches. *Food Chemistry,* 101**,** 1338-1345.

IGBEDIOH, S. O., OLUGBEMI, K. T. & AKPAPUNAM, M. A. 1994. Effects of processing methods on phytic acid level and some constituents in bambara groundnut (Vigna subterranea) and pigeon pea (Cajanus cajan). *Food Chemistry,* 50**,** 147-151.

IHEKORONYE, A. I. & NGODDY, P. O. 1985. Food carbohydrate. *Integrated Food science and Technology for the Tropics.* Macmillan.

JACOBS, H. & DELCOUR, J. A. 1998. Hydrothermal Modifications of Granular Starch, with Retention of the Granular Structure: A Review. *Journal of Agricultural and Food Chemistry,* 46**,** 2895-2905.

JACOBS, H., EERLINGEN, R. C., CLAUWAERT, W. & DELCOUR, J. A. 1995. Influence of Annealing on the Pasting Properties of Starches from Varying Botanical Sources. . *Cereal Chemistry***,** 480-487.

JAYAKODY, L. & HOOVER, R. 2008. Effect of annealing on the molecular structure and physicochemical properties of starches from different botanical origins–A review. *Carbohydrate Polymers,* 74**,** 691-703.

JIMÉNEZ, T. & MARTÍNEZ-ANAYA, M. A. 2001. Amylases and hemicellulases in breadmaking. Degradation by-products and potential relationship with functionality. *Revista de Agaroquimica y Tecnologia de Alimentos,* 7**,** 5-14.

JOSEPH, E., CRITES, S. G. & SWANSON, B. G. 1993. Microstructure of black, green and red gram. *Food structure,* 12**,** 3.

JOSEPH, G. A., DAVID, G. B. & ETIENNE, P. J. 2006. Seed storage and germination. *Medicago truncatula handbook.* France: Laboratoire des Interactions Plantes-Microorganismes (LIPM), CNRS-INRA, BP52627, 31326 Castanet-Tolosan Cedex, France.

KASOTE, D. M., NILEGAONKAR, S. S. & AGTE, V. V. 2014. Effect of different processing methods on resistant starch content and in vitro starch digestibility of some common Indian pulses. *Journal of Scientific & Industrial Research*
73**,** 541-546.

KAUR, M. & SANDHU, K. 2010a. Functional, thermal and pasting characteristics of flours from different lentil (Lens culinaris) cultivars. *Journal of Food Science and Technology,* 47**,** 273-278.

KAUR, M. & SANDHU, K. S. 2010b. In vitro digestibility, structural and functional properties of starch from pigeon pea (Cajanus cajan) cultivars grown in India. *Food Research International,* 43**,** 263-268.

KAUR, M., SINGH, N. & SODHI, N. S. 2005. Physicochemical, cooking, textural and roasting characteristics of chickpea (Cicer arietinum L.) cultivars. *Journal of Food Engineering,* 69**,** 511-517.

KAUSHAL, P., KUMAR, V. & SHARMA, H. K. 2012. Comparative study of physicochemical, functional, antinutritional and pasting properties of taro (Colocasia esculenta), rice (Oryza sativa) flour, pigeonpea (Cajanus cajan) flour and their blends. *LWT - Food Science and Technology,* 48**,** 59-68.

KHAN, A. R., ALAM, S., ALI, S., BIBI, S. & KHALIL, I. A. 2007. Dietary fiber profile of food legumes. *Sarhad Journal of Agriculture,* 23**,** 763.

KIGEL, J. 1999. Culinary and nutritional quality of Phaseolus vulgaris seeds as affected by environmental factors. *Biotechnologie, Agronomie, Société et Environnement,* 3**,** 205-209.

KIKUCHI, K., KOIZUMI, M., ISHIDA, N. & KANO, H. 2006. Water Uptake by Dry Beans Observed by Micro-magnetic Resonance Imaging. *Annals of Botany,* 98**,** 545-553.

KINYANJUI, P. K., NJOROGE, D. M., MAKOKHA, A. O., CHRISTIAENS, S., NDAKA, D. S. & HENDRICKX, M. 2015. Hydration properties and texture fingerprints of easy- and hard-to-cook bean varieties. *Food Science & Nutrition,* 3**,** 39-47.

KOKINI, J. L., HO, C.-T. & KARWE, M. V. 1992. *Food extrusion science and technology*, M. Dekker.

KUMAR, V., SINHA, A. K., MAKKAR, H. P. & BECKER, K. 2010. Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry,* 120**,** 945-959.

LAI, L. & KOKINI, J. 1991. Physicochemical changes and rheological properties of starch during extrusion (a review). *Biotechnology progress,* 7**,** 251-266.

LAI, L. S. & KOKINI, J. L. 1990. The effect of extrusion operating conditions on the on-line apparent viscosity of 98% Amylopectin (Amioca) and 70% Amylose (Hylon 7) corn starches during extrusion *Journal of Rheology,* 34**,** 1245-1266.

LAMPART-SZCZAPA, E. 2001. Legumes and Oilseed Protein. *In:* SIKORSKI, Z. E. (ed.) *Chemical and Functional properties of Food Proteins.* Lancaster , PA: Technomic.

LAWAL, O. S. 2011. Hydroxypropylation of pigeon pea (Cajanus cajan) starch: Preparation, functional characterizations and enzymatic digestibility. *LWT - Food Science and Technology,* 44**,** 771-778.

LIN, S., HSIEH, F. & HUFF, H. 1997. Effects of lipids and processing conditions on degree of starch gelatinization of extruded dry pet food. *LWT-Food Science and Technology,* 30**,** 754-761.

LIU, K., HUNG, Y.-C. & PHILLIPS, R. 1993a. Mechanism of hard-to-cook defect in cowpeas: verification via microstructure examination. *Food structure*.

LIU, K., MCWATTERS, K. H. & PHILLIPS, R. D. 1992. Protein insolubilization and thermal destabilization during storage as related to hard-to-cook defect in cowpeas. *Journal of Agricultural and Food Chemistry,* 40**,** 2483-2487.

LIU, K., PHILLIPS, R. D. & MCWATTERS, K. H. 1993b. Mechanism of pectin changes during soaking and heating as related to hard-to-cook defect in cowpeas. *Journal of Agricultural and Food Chemistry,* 41**,** 1476-1480.

LIU, Y., HSIEH, F., HEYMANN, H. & HUFF, H. 2000. Effect of Process Conditions on the Physical and Sensory Properties of Extruded Oat-Corn Puff. *Journal of Food Science,* 65**,** 1253-1259.

LLUCH, A. M., PEREZ-MUNUERA, I. & HERNANDO, I. 2001. Protein in Food Structure. *In:* SIKORSKI, Z. E. (ed.) *Chemical and Functional Properties of Food Proteins.* Lancaster, Pennsylvania: Technomic.

LOPEZ, H. W., LEENHARDT, F., COUDRAY, C. & REMESY, C. 2002. Minerals and phytic acid interactions: is it a real problem for human nutrition? *International journal of food science & technology,* 37**,** 727-739.

MA, C. Y. & HARWALKAR, V. 1988. Studies of thermal denaturation of oat globulin by differential scanning calorimetry. *Journal of Food Science,* 53**,** 531-534.

Maenz, D.D. 2001. Enzymatic characteristics of phytate as they relate to their use in animal feeds, In Bedford, M.R.and Partridge,G.C. (eds) Enzymes in Animal Nutrition. CAB Publishing, Oxford 61-64.

MAHADEVAMMA, S., PRASHANTH, K. H. & THARANATHAN, R'"]',. 2003. Resistant starch derived from processed legumes—purification and structural characterization. *Carbohydrate polymers,* 54**,** 215-219.

MANINDER, K., SANDHU, K. S. & SINGH, N. 2007. Comparative study of the functional, thermal and pasting properties of flours from different field pea (Pisum sativum L.) and pigeon pea (Cajanus cajan L.) cultivars. *Food chemistry,* 104**,** 259-267.

MARBACH, I. & MAYER, A. M. 1974. Permeability of Seed Coats to Water as Related to Drying Conditions and Metabolism of Phenolics. *Plant Physiology,* 54**,** 817-820.

MARTÍN-CABREJAS, M. A., ESTEBAN, R. M., PEREZ, P., MAINA, G. & WALDRON, K. W. 1997. Changes in physicochemical properties of dry beans (Phaseolus vulgaris L.) during long-term storage. *Journal of Agricultural and Food Chemistry,* 45**,** 3223-3227.

MARTÍN-CABREJAS, M. A., JAIME, L., KARANJA, C., DOWNIE, A. J., PARKER, M. L., LOPEZ-ANDREU, F. J., MAINA, G., ESTEBAN, R. M., SMITH, A. C. & WALDRON, K. W. 1999. Modifications to Physicochemical and Nutritional Properties of Hard-To-Cook Beans (Phaseolus vulgaris L.) by Extrusion Cooking. *Journal of Agricultural and Food Chemistry,* 47**,** 1174-1182.

MASKUS, H. & ARNTFIELD, S. 2015. Extrusion Processing and Evaluation of an Expanded, Puffed Pea Snack Product. *Nutrition and Food science,* 5**,** 1-6.

MATTHEWS, S. A. & POWELL, A. 2006. Electrical Conductivity Vigour Test: Physiological Basis and Use. *Seed Testing International,* 131**,** 32-35.

MATTSON, S. 1946. The cookability of yellow peas. A colloid-chemical and biochemical study. *Acta Agriculturae Suecana,* 2**,** 185-231.

MAYZELLE, M. & BELL, M. 2014. Pigeon Pea Nutritional Benefits. *In:* UNIVERSITY OF CALIFORNIA, D. (ed.) *USDA Nutrient Database; NRCS Plant.*

MENG, G. T. & MA, C. Y. 2001. Thermal properties of Phaseolus angularis (red bean) globulin. *Food Chemistry,* 73**,** 453-460.

MILEWSKI, S. 2001. Protein Structure and Physicochemical Properties. *In:* SIKORSKI, Z. E. (ed.) *Chemical and Functional Properties of Food Protein.* Lancaster, PA: Technomic.

MOÏSE, J. A., HAN, S., GUDYNAITĘ-SAVITCH, L., JOHNSON, D. A. & MIKI, B. L. A. 2005. SEED COATS: STRUCTURE, DEVELOPMENT, COMPOSITION, AND BIOTECHNOLOGY. *In Vitro Cellular and Developmental Biology - Plant,* 41**,** 620-644.

MORARU, C. & KOKINI, J. 2003. Nucleation and expansion during extrusion and microwave heating of cereal foods. *Comprehensive Reviews in Food Science and Food Safety,* 2**,** 147-165.

MORRIS, V. J. 1990. Starch gelation and retrogradation. *Trends in Food Science & Technology,* 1**,** 2-6.

MORRISON, W. R., TESTER, R. F., SNAPE, C. E., LAW, R. & GIDLEY, M. J. 1993. Swelling and gelatinization of cereal starches. IV. Some effects of lipid-complexed. *Cereal Chem,* 70**,** 385-391.

MOSCICKI, L. 2011. *Extrusion-cooking techniques: applications, theory and sustainability*, John Wiley & Sons.

MOSCICKI, L. & ZUILICHEM, D. J. V. 2011. Extrusion Cooking and Related Technique. *In:* KI, L. (ed.) *Extrusion-cooking techniques: applications, theory and sustainability.* Weinheim: John Wiley & Sons.

MULLIN, W. J. & XU, W. 2001. Study of soybean seed coat components and their relationship to water absorption. *Journal of agricultural and food chemistry,* 49**,** 5331-5335.

MWASARU, M. A., MUHAMMAD, K., BAKAR, J. & MAN, Y. B. C. 1999. Effects of isolation technique and conditions on the extractability, physicochemical and functional properties of pigeonpea (Cajanus cajan) and cowpea (Vigna unguiculata) protein isolates. I. Physicochemical properties. *Food Chemistry,* 67**,** 435-443.

NASAR-ABBAS, S. M., PLUMMER, J. A., SIDDIQUE, K. H., WHITE, P., HARRIS, D. & DODS, K. 2008. Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening. *LWT-Food Science and Technology,* 41**,** 1260-1267.

NOAH, L., GUILLON, F., BOUCHET, B., BULÉON, A., MOLIS, C., GRATAS, M. & CHAMP, M. 1998. Digestion of Carbohydrate from White Beans (Phaseolus vulgaris L.) in Healthy Humans. *The Journal of Nutrition,* 128**,** 977-985.

OBOH, H., OSAGIE, A. & OMOTOSHO, A. 2010. Glycemic response of some boiled legumes commonly eaten in Nigeria. *Diabetologia Croatica,* 39**,** 125-131.

OBOH, H. A., MUZQUIZ, M., BURBANO, C., CUADRADO, C., PEDROSA, M. M., AYET, G. & OSAGIE, A. U. 2000. Effect of soaking, cooking and germination on the oligosaccharide content of selected Nigerian legume seeds. *Plant Foods for Human Nutrition,* 55**,** 97-110.

ODENY, D. A. The potential of pigeonpea (Cajanus cajan (L.) Millsp.) in Africa. Natural resources forum, 2007. Wiley Online Library, 297-305.

OKE, D. G. 2014. Proximate and Phytochemical Analysis of Cajanus Cajan (Pigeon Pea) Leaves. *Chemical Science Transactions,* 3**,** 1172-1178.

OKPALA, L., OKOLI, E. & UDENSI, E. 2013. Physico-chemical and sensory properties of cookies made from blends of germinated pigeon pea, fermented sorghum, and cocoyam flours. *Food Science & Nutrition,* 1**,** 8-14.

OLOGHOBO, A. & FETUGA, B. 1984. Distribution of phosphorus and phytate in some Nigerian varieties of legumes and some effects of processing. *Journal of Food Science,* 49**,** 199-201.

ONIMAWO, I. & AKPOJOVWO, A. 2006. Toasting (dry heat) and nutrient composition, functional properties and antinutritional factors of pigeon pea (Cajanus cajan) flour. *Journal of food processing and preservation,* 30**,** 742-753.

ONWUKA, G. I. 2006. SOAKING, BOILING AND ANTINUTRITIONAL FACTORS IN PIGEON PEAS (CAJANUS CAJAN) AND COWPEAS (VIGNA UNGUICULATA). *Journal of Food Processing and Preservation,* 30**,** 616-630.

OZCAN, S. & JACKSON, D. S. 2005. Functionality behavior of raw and extruded corn starch mixtures.

PAI, D. A., BLAKE, O. A., HAMAKER, B. R. & CAMPANELLA, O. H. 2009. Importance of extensional rheological properties on fiber-enriched corn extrudates. *Journal of Cereal Science,* 50**,** 227-234.

PAIVA, É. A. S., LEMOS-FILHO, J. P. & OLIVEIRA, D. M. T. 2006. Imbibition of Swietenia macrophylla (Meliaceae) Seeds: The Role of Stomata. *Annals of Botany,* 98**,** 213-217.

PARADA, J. & AGUILERA, J. M. 2009. In vitro digestibility and glycemic response of potato starch is related to granule size and degree of gelatinization. *Journal of Food Science,* 74**,** E34-E38.

PARKER, R. & RING, S. 2001. Aspects of the physical chemistry of starch. *Journal of Cereal Science,* 34**,** 1-17.

PELEG, M. 1988. An empirical model for the description of moisture sorption curves. *Journal of Food science,* 53**,** 1216-1217.

PÉREZ, S. & BERTOFT, E. 2010. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch-Stärke,* 62**,** 389-420.

PLHAK, L. C., CALDWELL, K. B. & STANLEY, D. W. 1989. Comparison of Methods Used to Characterize Water Imbibition in Hard-To-Cook Beans. *Journal of Food Science,* 54**,** 326-329.

PLHAK, L. C., STANLEY, D. W., HOHLBERG, A. I. & AGUILERA, J. M. 1987. Hard-to-Cook Defect in Black Beans — Effect of Pretreatment and Storage Condition on Extractable Phenols and Peroxidase Activity. *Canadian Institute of Food Science and Technology Journal,* 20**,** 378-382.

POUTANEN, K. 1997. Enzymes: An important tool in the improvement of the quality of cereal foods. *Trends in Food Science & Technology,* 8**,** 300-306.

POWELL, A. A. 1986. Cell membranes and seed leachate conductivity in relation to the quality of seed for sowing. *Journal of Seed Technology***,** 81-100.

PRAKASH, A. S., PEREIRA, T. N., REILLY, P. E. B. & SEAWRIGHT, A. A. 1999. Pyrrolizidine alkaloids in human diet. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 443**,** 53-67.

RAFIQ, S. I., SINGH, S. & SAXENA, D. 2016. Effect of alkali-treatment on physicochemical, pasting, thermal, morphological and structural properties of Horse Chestnut (Aesculus indica) starch. *Journal of Food Measurement and Characterization,* 10**,** 676-684.

RAIZ, M. N. 2001. Selecting the right extruder. *In:* ROBIN, G. (ed.) *Extrusion cooking: technologies and applications.* Woodhead publishing.

RALET, M. C., DELLA VALLE, G. & THIBAULT, J. F. 1993. Raw and extruded fibre from pea hulls.Part 1:composition and physicochemical properties. *Carbohydrate Polymers,* 20**,** 17-23.

RAMPERSAD, R., BADRIE, N. & COMISSIONG, E. 2003. Physico-chemical and Sensory Characteristics of Flavored Snacks from Extruded Cassava/Pigeonpea Flour. *Journal of food science,* 68**,** 363-367.

RATNAYAKE, R. 2006. A new insight into the phase transition processes of food starches.

RATNAYAKE, W. S. & JACKSON, D. S. 2007. A new insight into the gelatinization process of native starches. *Carbohydrate Polymers,* 67**,** 511-529.

RIETJENS, I. M., MARTENA, M. J., BOERSMA, M. G., SPIEGELENBERG, W. & ALINK, G. M. 2005. Molecular mechanisms of toxicity of important food-borne phytotoxins. *Molecular nutrition & food research,* 49**,** 131-158.

ROBIN, F., DAUTREMONT, C. & CHANVRIER, H. 2014. Winning consumer preference via extrusion cooking of nutritious cereals. *New Food.* Brasted, Kent: Russell Publishing Limited,.

ROBIN, F., DUBOIS, C., PINEAU, N., SCHUCHMANN, H. P. & PALZER, S. 2011. Expansion mechanism of extruded foams supplemented with wheat bran. *Journal of food engineering,* 107**,** 80-89.

ROBIN, F., ENGMANN, J., PINEAU, N., CHANVRIER, H., BOVET, N. & DELLA VALLE, G. 2010. Extrusion, structure and mechanical properties of complex starchy foams. *Journal of Food Engineering,* 98**,** 19-27.

ROBIN, F., SCHUCHMANN, H. P. & PALZER, S. 2012. Dietary fiber in extruded cereals: Limitations and opportunities. *Trends in Food Science & Technology,* 28**,** 23-32.

ROSKHRUA, P., TRAN, T., CHAIWANICHSIRI, S., KUPONGSAK, S. & PRADIPASENA, P. 2014. Physicochemical properties of thermal alkaline treated pigeonpea (Cajanus cajan L.) flour. *Food Science and Biotechnology,* 23**,** 381-388.

ROSS, K. A., ARNTFIELD, S. D. & CENKOWSKI, S. 2013. A Polymer Science Approach to Physico-Chemical characterization and Procesing of Pulse Seeds. Croatia: Intech.

ROUT, B., SAHOO, S. & SENAPATI, P. K. 2007. Effect of pre-milling treatment on protein and carbohydrate content in tribal pulses. *Indian Journal of Traditional Knowledge*
6**,** 69-71.

RUIZ-RUIZ, J., MARTÍNEZ-AYALA, A., DRAGO, S., GONZÁLEZ, R., BETANCUR-ANCONA, D. & CHEL-GUERRERO, L. 2008. Extrusion of a hard-to-cook bean (Phaseolus vulgaris L.) and quality protein maize (Zea mays L.) flour blend. *LWT - Food Science and Technology,* 41**,** 1799-1807.

SAARELA, M. 2011. *Functional Foods: Concept to Product*, Elsevier Science.

SAIO, K. & MONMA, M. 1993. Microstructural approach to legume seeds for food uses. *Food structure,* 12**,** 6.

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

SAXENA, K. B., KUMAR, R. V. & SULTANA, R. 2010. Quality nutrition through pigeonpea—a review. *Health,* 2**,** 1335-1344.

SEENA, S. & SRIDHAR, K. R. 2005. Physicochemical, functional and cooking properties of under explored legumes, Canavalia of the southwest coast of India. *Food Research International,* 38**,** 803-814.

SHAFAEI, S. M., MASOUMI, A. A. & ROSHAN, H. 2014. Analysis of water absorption of bean and chickpea during soaking using Peleg model. *Journal of the Saudi Society of Agricultural Sciences*.

SHIGA, T. M., CORDENUNSI, B. R. & LAJOLO, F. M. 2009. Effect of cooking on non-starch polysaccharides of hard-to-cook beans. *Carbohydrate Polymers,* 76**,** 100-109.

SHOMER, I., PASTER, N., LINDNER, P. & VASILIVER, R. 1990. The role of cell wall structure in the hard-to-cook phenomenon in beans (Phaseolus vulgaris L.). *Food Structure,* 9**,** 10.

SIKORSKI, Z. E. 2001a. Chemical reactions in Proetin in Food System. *In:* SIKORSKI, Z. E. (ed.) *Chemical and Functional Properties of Food Protein.* Lancaster,PA: Technomic.

SIKORSKI, Z. E. 2001b. The Role of Nitrogenous Compounds in Food Quality. *In:* SIKORSKI, Z. E. (ed.) *Chemical and Functional Properties of Food Protein.* Lancaster,P.A: Technomic

SILVA, E. M. M. D., ASCHERI, J. L. R., CARVALHO, C. W. P. D., TAKEITI, C. Y. & BERRIOS, J. D. J. 2014. Physical characteristics of extrudates from corn flour and dehulled carioca bean flour blend. *LWT - Food Science and Technology,* 58**,** 620-626.

SINGH, B. & KULSHRESTHA, S. 1987. Kinetics of water sorption by soybean and pigeonpea grains. *Journal of Food Science,* 52**,** 1538-1541.

SINGH, N., KAUR, M., SANDHU, K. S. & SODHI, N. S. 2004. Physicochemical, cooking and textural characteristics of some Indian black gram (Phaseolus mungo L) varieties. *Journal of the Science of Food and Agriculture,* 84**,** 977-982.

SINGH, N., NAKAURA, Y., INOUCHI, N. & NISHINARI, K. 2008a. Structure and Viscoelastic Properties of Starches Separated from Different Legumes. *Starch - Stärke,* 60**,** 349-357.

SINGH, N., NAKAURA, Y., INOUCHI, N. & NISHINARI, K. 2008b. Structure and viscoelastic properties of starches separated from different legumes. *Starch‐Stärke,* 60**,** 349-357.

SINGH, S., SINGH, N. & MACRITCHIE, F. 2011. Relationship of polymeric proteins with pasting, gel dynamic-and dough empirical-rheology in different Indian wheat varieties. *Food Hydrocolloids,* 25**,** 19-24.

SINGH, U., JAMBUNATHAN, R. & NARAYANAN, A. 1980. Biochemical changes in developing seeds of pigeonpea (Cajanus cajan). *Phytochemistry,* 19**,** 1291-1295.

SINGH, U., JAMBUNATHAN, R., SAXENA, K. & SUBRAHMANYAM, N. 1990. Nutritional quality evaluation of newly developed high-protein genotypes of pigeonpea (Cajanus cajan). *Journal of the Science of Food and Agriculture,* 50**,** 201-209.

SLADE, L. & LEVINE, H. 1988. Non-equilibrium melting of native granular starch: Part I. Temperature location of the glass transition associated with gelatinization of A-type cereal starches. *Carbohydrate Polymers,* 8**,** 183-208.

SLADE, L. & LEVINE, H. 1995. Glass transitions and water–food
structure interactions. *Advances in Food and Nutrition Research,* 38**,** 103-126.

SOETAN, K. & OYEWOLE, O. 2009. The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science,* 3**,** 223-232.

SOUZA, F. H. D. D. & MARCOS-FILHO, J. 2001. The seed coat as a modulator of seed-environment relationships in Fabaceae. *Brazilian Journal of Botany,* 24**,** 365-375.

SPIGNO, G. & DE FAVERI, D. M. 2004. Gelatinization kinetics of rice starch studied by non-isothermal calorimetric technique: influence of extraction method, water concentration and heating rate. *Journal of Food Engineering,* 62**,** 337-344.

STANLEY, D. W. & AGUILERA, J. M. 1985. A REVIEW OF TEXTURAL DEFECTS IN COOKED RECONSTITUTED LEGUMES —THE INFLUENCE OF STRUCTURE AND COMPOSITION. *Journal of Food Biochemistry,* 9**,** 277-323.

STEEL, C. J., SCHMIELE, M., LEORO, M. G. V., FERREIRA, R. E. & CHANG, Y. K. 2012. *Thermoplastic extrusion in food processing*, Citeseer.

SVENSSON, E. & ELIASSON, A.-C. 1995. Crystalline changes in native wheat and potato starches at intermediate water levels during gelatinization. *Carbohydrate Polymers,* 26**,** 171-176.

SWANSON, B. G., HUGHES, J. S. & RASMUSSEN, H. P. 1985. Seed Microstructure: Review of Water Imbibition in Legumes. *Food Structure* 4**,** 115-124.

TAKAYA, T., SANO, C. & NISHINARI, K. 2000. Thermal studies on the gelatinisation and retrogradation of heat–moisture treated starch. *Carbohydrate Polymers,* 41**,** 97-100.

TAYEB, J., DELLA VALLE, G. & VERGNES , B. 1992. Simulation of Transport Phenomena in Twin Screw extruders *In:* KOKINI, J. L., HO, C.-T. & KARWE, M. V. (eds.) *Food extrusion science and technology.* New york: M. Dekker.

TESTER, R. F. & DEBON, S. J. 2000. Annealing of starch—a review. *International journal of biological macromolecules,* 27**,** 1-12.

TESTER, R. F. & SOMMERVILLE, M. D. 2003. The effects of non-starch polysaccharides on the extent of gelatinisation, swelling and α-amylase hydrolysis of maize and wheat starches. *Food Hydrocolloids,* 17**,** 41-54.

TIWARI, B. K., JAGANMOHAN, R., VENKATACHALAPATHY, N., TITO ANAND, M., SURABI, A. & ALAGUSUNDARAM, K. 2010. Optimisation of hydrothermal treatment for dehulling pigeon pea. *Food Research International,* 43**,** 496-500.

TIWARI, B. K. & SINGH, N. 2012. *Pulse Chemistry and Technology*, Royal Society of Chemistry.

TIWARI, B. K., TIWARI, U., JAGAN MOHAN, R. & ALAGUSUNDARAM, K. 2008. Effect of Various Pre-treatments on Functional, Physiochemical, and Cooking Properties of Pigeon pea (Cajanus cajan L). *Food Science and Technology International,* 14**,** 487-495.

VALLE, G. D., QUILLIEN, L. & GUEGUEN, J. 1994. Relationships between processing conditions and starch and protein modifications during extrusion-cooking of pea flour. *Journal of the Science of Food and Agriculture,* 64**,** 509-517.

VAN DER SMAN, R. G. M. & MEINDERS, M. B. J. 2011. Prediction of the state diagram of starch water mixtures using the Flory-Huggins free volume theory. *Soft Matter,* 7**,** 429-442.

VANDEPUTTE, G. E. & DELCOUR, J. A. 2004. From sucrose to starch granule to starch physical behaviour: a focus on rice starch. *Carbohydrate Polymers,* 58**,** 245-266.

VARZAKAS, T. & TZIA, C. 2015. *Handbook of Food Processing: Food Preservation*, CRC Press.

VASISHTHA, H., SRIVASTAVA, R. P. & VERMA, P. 2012. Milling and cooking effects on dietary fibre, protein and lectins of pigeonpea (Cajanus cajan). *Current Advances in Agricultural Sciences* 4**,** 134-138.

VERTUCCI, C. W. & LEOPOLD, A. C. 1987. Water Binding in Legume Seeds. *Plant Physiology,* 85**,** 224-231.

WALDRON, K. W., PARKER, M. L. & SMITH, A. C. 2003. Plant Cell Walls and Food Quality. *Comprehensive Reviews in Food Science and Food Safety,* 2**,** 128-146.

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

WANG, N., HATCHER, D. W., WARKENTIN, T. D. & TOEWS, R. 2010. Effect of cultivar and environment on physicochemical and cooking characteristics of field pea (Pisum sativum). *Food Chemistry,* 118**,** 109-115.

WANG, S. & COPELAND, L. 2012. Effect of alkali treatment on structure and function of pea starch granules. *Food Chemistry,* 135**,** 1635-1642.

WILLIAMS, P. C., NAKOUL, H. & SINGH, K. 1983. Relationship between cooking time and some physical characteristics in chickpeas (Cicer arietinum L.). *Journal of the Science of Food and Agriculture,* 34**,** 492-496.

WINK, M. 2010. Introduction: biochemistry, physiology and ecological functions of secondary metabolites. *Annual Plant Reviews Volume 40: Biochemistry of Plant Secondary Metabolism, Second Edition***,** 1-19.

WONGDECHSAREKU, S. & KONGKIATTIKAJORN, J. 2010. Storage time affects storage proteins and volatile compounds, and pasting behavior of milled rice. *KKU Res J,* 15**,** 852-862.

YADAV, S. P. 1984. Physical and chemical analyses of pigeonpea (Cajanus cajan) cultivars and F1 crosses. *Journal of the Science of Food and Agriculture,* 35**,** 833-836.

YOUSIF, A. M., DEETH, H. C., CAFFIN, N. A. & LISLE, A. T. 2002. Effect of Storage Time and Conditions on the Hardness and Cooking Quality of Adzuki (Vigna angularis). *LWT - Food Science and Technology,* 35**,** 338-343.

YU, L., RAMASWAMY, H. S. & BOYE, J. 2013. Protein rich extruded products prepared from soy protein isolate-corn flour blends. *LWT - Food Science and Technology,* 50**,** 279-289.

ZHOU, M., ROBARDS, K., GLENNIE-HOLMES, M. & HELLIWELL, S. 1999. Oat lipids. *Journal of the American Oil Chemists' Society,* 76**,** 159-169.

ZHOU, Y., HOOVER, R. & LIU, Q. 2004. Relationship between α-amylase degradation and the structure and physicochemical properties of legume starches. *Carbohydrate Polymers,* 57**,** 299-317.

ZOBEL, H. F. 1988a. Molecules to Granules: A Comprehensive Starch Review. *Starch - Stärke,* 40**,** 44-50.