4 GENERAL DISCUSSION

The discussion will first examine the strengths and weaknesses of the experimental design and some of the methodologies used in the study. Secondly, the mechanisms involved in micronisation-induced reduction in cooking time and increase in splitting of cowpeas will be scrutinised. A thesis on how micronisation of moisture-conditioned cowpeas to mild temperatures (130 °C and 153 °C) was more effective in reducing cooking time of whole seeds and produced flour with different functionality than micronisation to higher temperatures (170 °C) will be put forward.

4.1 Critical review of experimental design and methodologies

The purpose of this study was to investigate the underlying effects of micronisation (41 % moisture and infrared heating) on cowpea seed structure and the major constituents namely protein and starch and how these may affect the cooking characteristics of whole cowpea seeds and functional properties of the resultant flour.

In order to include the possible differences due to variety, nine cowpea lines were initially screened to select two divergent varieties for the study. Variations in cooking characteristics of cowpea varieties have been reported by several researchers (Akinyele *et al*., 1986; Taiwo *et al*., 1997b), hence it was decided to use two divergent varieties, to determine if the effect of the micronisation (41 % moisture and 153 °C) process on these two varieties would be different. The two selected varieties (Bechuana white and Var. 462) had similar cooking times, but had different hydration properties during soaking, as well as different textural and splitting properties during cooking. However, the varieties were produced in different areas in a single season, such that it would not be possible to fully attribute the differences exhibited by these varieties to varietal (genetic and phenotypic) effects only. Growing location has been shown to have an effect on cooking times of freshly harvested beans (Proctor & Watts, 1987b). However the existing differences in seed density and hydration properties during soaking and cooking of the cowpeas were instrumental in explaining the variations observed in cooked seed texture

and splitting of the unmicronised seeds. Following the micronisation (41 % moisture and 153 °C) treatment, it was evident that there were slight differences in the magnitude of the effect of micronisation (41 % moisture and 153 °C) on some physicochemical properties of the cowpeas. It is possible that the variety effect during micronisation (41 % moisture and 153 °C) would have been more evident if varieties that differed substantially in cooking time had been used.

Infrared (IR) is a type of non ionising radiation and micronisation utilises parts of the near (760 – 3000 nm) and mid (3000-30,000 nm) regions of IR. A tabletop microniser used in this study for the micronisation of samples comprised of 3 infrared lamps (2 kW each) that radiated energy on stationary cowpeas samples. Infra red lamps emit energy at shorter wavelengths (around the 1000 nm wavelength) than gas fired micronisers, which have wavelengths in the range of 1800 – 3400 nm (Cenkowski & Sosulski, 1998). The peak wavelength for infrared lamp emission is reported to be 1150 nm (Cenkowski & Sosulski, 1998) and have photon energy of around 1.9 x 10^{-19} J/photon at 1000 nm (Schieke, Schroeder & Krutmann, 2003). Vibrating troughs or belts have been used in pilot scale equipment to facilitate uniform exposure of the samples to the infrared radiation (Cenkowski, Hong, Scanlon & Arntfield, 2003). Although the cowpeas were micronised from one direction only, the micronisation treatment was still effective since the cowpeas used in this study were small seeded. It has been shown by Fasina *et al.* (2001) that it was easier to uniformly micronise small seeded legumes such as lentils and split peas than larger seeded legumes such as pinto beans.

The cowpeas were tempered to 41 % moisture to facilitate partial starch gelatinisation and protein denaturation during micronisation. The moisture content used in this research work was high to be economically feasible in a commercial setting. Due to the high moisture content it would be necessary to dry the cowpeas hence increasing the energy requirement for the process. Arntfield *et al.* (1997) demonstrated that lower moisture contents (29 - 33 %) were effectively used in reducing the cooking time of lentils. Therefore process optimisation studies would be required to identify the optimum moisture content for maximum micronisation effect and optimal residual moisture.

Since micronisation (41 % moisture and infrared heating) of samples was conducted in an open system, there was loss of moisture as the process progressed. This implies that moisture content of the seeds was not constant throughout the processing time. Towards the end of the micronisation process, the seeds were micronised with limited moisture especially for the 170 °C treatment. During the first 3 min of micronisation the moisture content of the seeds declined from 41 % to approximately 25 %, and 12 % in 6 min ending with 5 % at the end of micronisation (8 min) when 170 °C was attained. This means that during the last 2 min of micronisation the samples had less than 12 % moisture.

To study the effect of low and high micronisation temperatures, the moisture-conditioned (41 %) cowpeas were initially micronised to 130 and 153 °C. However there were no significant differences in the cooking characteristics of the two treatments, hence a higher temperature (170 °C) was included in the design. However, Var. 462 could not be micronised to 170 °C without burning possibly due to differences in optical and thermal properties such as thermal conductivity of the seeds. Var. 462 was maroon in colour and would possibly absorb more of the IR energy that was being transferred than the lighter coloured Bechuana white. Therefore Var. 462 cowpeas could not be used in the experiments involving the high temperature of 170 °C. Bulk density is one of the factors that affect thermal conductivity and it has been reported that thermal conductivity in cowpea flour increased with increasing bulk density (Taiwo, Akanbi & Ajibola, 1996). Var. 462 was less dense than Bechuana white cowpeas and would have relatively lower thermal conductivity. However during infrared heating, it has been indicated that the absorption of infrared energy increases with thickness (aerated distance) and relative humidity of the air (Mohsenin, 1984). Therefore, Var. 462 that had more air pockets at 41 % moisture probably absorbed infrared energy more easily than the compact Bechuana white seeds.

Cooking time is one of the food quality criterions that are used in crop improvement programs to evaluate the food quality of whole cowpea seeds (Ehlers & Hall, 1997). Cooking time is defined as the time required for cowpeas to attain a level of softness that

is acceptable for consumption (Proctor & Watts, 1987a). In this study, a Mattson bean cooker (MBC) and texture measurements using a TA-XT2 texture analyser (Stable Micro Systems, Goldaming, UK) with a craft blade attachment were used to predict cooking time. The use of the MBC was time consuming and required constant attention throughout the cooking period for each sample. Use of automated bean cookers would address the problem of attending to the samples throughout the cooking process, although automated models are relatively expensive. Apart from the high time requirement for measurements, cooking time of cowpeas determined in this study most probably did not reflect the actual cooking time for the consumer. Proctor and Watts (1987a) demonstrated that lighter rods (49.75 g) provided the best indication of cooking time that correlated with sensory perception of a cooked bean. The rods used in this study were 90 g and would underestimate the actual cooking time. However, the MBC values obtained in this study still provided a basis for comparison between the treatments. It is difficult to compare results from different studies obtained using a MBC due to differences in the application of the procedure. Differences have been reported in connection with pre-treatment of the samples with regards to soaking (i.e. soaking vs. no soaking, length of soaking time), weight of rods (38 to 90 g) (Akinyele *et al*., 1986; Wang *et al*., 2003) and the number of rods used (ranging from 6 to 100) (Abdul-Kadir *et al*., 1990; DeToro, 1993). In addition, variations have also been reported in the way cooking time is recorded, i.e. whether the samples are added to boiling water or cold water and at what stage cooking time is determined (i.e. 50, 80 or 100 percentile) (Akinyele *et al*., 1986; Berrios, Swanson & Cheong, 1999).

The use of the texture analyser with a blade attachment proved to be difficult in measuring seed hardness at different stages of cooking. The dry uncooked seeds were fractured upon impact, and hence the data could not be included in the texture analysis, since this was a different textural property from the hard to mushy texture of the seeds measured during cooking. It was observed during the preliminary stage of this research that there was wide variation in textural measurements of the cowpea seeds during the first 15 min of cooking. These variations were due to differences in hydration rate of individual seeds within a sample. In addition, cowpeas are biological material; and they

have inherent variations between individual seeds. These inherent variations were evident in the texture of seeds at the same cooking time interval contributing to high standard deviations of the means. In order to address this problem, the number of cowpea seeds measured per sample was increased in subsequent experiments and this helped in increasing the degrees of freedom for variance, hence reducing the sample variance. The MBC and the texture measurements measured cooking time as a function of texture only, but the cooked state of cowpeas involves other sensory attributes such as flavour that has to be developed to an acceptable level during cooking (Proctor & Watts 1987a; Phadi, 2004). In order to capture detailed textural characteristics of the cowpeas, a descriptive sensory panel could have been used. The results from this research show that moisture-conditioned and micronised cowpeas were softer than unmicronised seeds during cooking. This information does not elaborate on the mouthfeel aspects of the texture (i.e. mushy, chewy, grainy and seed coat residues). These attributes will eventually have an effect on the subsequent performance of the product with the consumer.

Modern analytical techniques were used to explore the micronisation-induced changes in physicochemical and structural properties of the cowpea seeds, starch and protein. These changes helped to explain the reduction in cooking time and splitting observed in moisture-conditioned and micronised cowpeas as well as the modified functionality of the flour. To study the structural differences between the two cowpea varieties and the subsequent changes following micronisation (41 % moisture, 130 and 153, 170 °C) and cooking, microscopic techniques (light microscopy, scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM)) were employed. Differential scanning calorimetry (DSC) was used to study the thermal properties of the flour and isolated starch, while GP-HPLC was used to explore the possibility of starch depolymerisation in the flour from M-170 °C cowpeas. SDS-PAGE and fluorescence spectroscopy were used to study the nature of micronisation-induced protein denaturation in isolated protein-rich fractions.

SEM has been used successfully in this study and by other researchers to study the structural characteristics of cowpeas at different stages of soaking and cooking (Sefa-

Dedeh *et al*., 1978; Liu *et al*., 1993a; Phadi, 2004). In SEM, electrons serve the same purpose as light in light microscopy. The electrons are thus focussed on a specimen to form an image. The prime advantage of using electrons is that an electron has a much shorter wavelength than light and therefore a potentially much greater resolving power. Because electrons do not travel very far in air, the entire microscope column must be in a high vacuum. Due to the vacuum environment, the specimens had to be fixed and fully dehydrated before examination (Kalab, Allan-Wojtas & Miller, 1995). Due to fixing and dehydration of the samples in preparation for SEM there may be artefacts that have to be considered when observing and interpreting the micrographs. Since SEM requires drying of the samples, it could not be used to observe cooked samples that had been treated with enzymes to identify some of the material that was observed outside the parenchyma cells of cooked cotyledon. However, through the use of ESEM the cooked cowpea specimens were observed in their native form without fixation. Although the images were not as sharp as those obtained using the SEM, it was still possible to observe the structural changes in the cowpea cotyledon following cooking and enzyme treatment with pectinase and proteinase. The use of the enzymes to digest the material outside the parenchyma cells effectively verified the presence of pectic and protein material outside the parenchyma cells of cooked seeds. ESEM is a variable pressure SEM that enables visualization of uncoated, moist, dry or oily samples in a gaseous atmosphere in a vacuum range of 1-20 Torr (McDonough & Rooney, 1999). Water vapour is the gas of choice for samples that have to be viewed under a constant state of hydration. The resolution of the ESEM can be high but because of the gaseous environment inside the sample chamber, the pictures often lack the clear focus that is found in traditional SEM images (Roman-Gutierrez, Guilbert & Cuq, 2002). Thus through the use of SEM and ESEM it was possible to study the cowpea cotyledon structure from the raw seeds up to the cooked samples of both the unmicronised and micronised (moisture-conditioned, infrared heated) cowpeas. The use of SEM was successful and instrumental in observing the fissures that developed in the seed coat, cotyledon as well as parenchyma cells. This information was used to explain the increased hydration rate during soaking and cooking, as well as splitting in micronised (moisture-conditioned, infrared heated) seeds.

Bright field and polarised light microscopy was used to examine cowpea flour from unmicronised and micronised (M-130 °C and M-170 °C) seeds to observe structural differences that might explain the severe reduction in pasting properties of flour from the M-170 °C cowpeas. Although the magnification for light microscopy is modest in comparison to electron microscopy, it still proved useful in observing the change in birefringence of the starch granules and the presence of intact cells in flour from micronised (M-130 °C and M-170 °C) seeds. With the aid of acid Fuchsin and Congo red stains, light microscopy showed that micronised flours contained intact parenchyma cells, which had protein and damaged starch on the surface. Acid Fuchsin is used to stain protein, while Congo red stains β- glucans, which include damaged starch (Autio & Salmenkallio-Marttila, 2001).

Micronisation-induced changes in cowpea starch and protein were studied using differential scanning calorimetry (DSC). The technique has been used to study thermo properties of cowpea flour (Henshaw *et al*., 2003), cowpea starch isolated from untreated flour and γ-irradiated flour and paste (Abu *et al*., 2006b) and protein extracted from untreated cowpea (Horax *et al*., 2004a; Abu *et al*., 2006a) and from γ-irradiated flour and paste (Abu *et al*., 2006a). DSC is a thermal analytical technique for monitoring changes in physical or chemical properties of food materials as a function of temperature by detecting the heat changes associated with such processes (Kolbe *et al*., 1999). In DSC, the measuring principle is to compare the rate of heat flow to the sample and to an inert material, which are heated or cooled at the same rate. Changes in the sample associated with absorption or evolution of heat cause a change in the differential heat flow, which is then recorded as a peak. The area under the peak is directly proportional to the enthalpic change and its direction indicates whether the thermal event was endothermic or exothermic (Billiaderis, 1983).

In the present study, DSC was conducted to study the thermal properties of cowpea flour and starch isolated from the unmicronised and micronised (41 % moisture, 130, and 170 °C) seeds. The DSC thermograms for the flour from untreated seeds showed the presence of both the starch gelatinisation and protein denaturation endotherms. In

micronised (M-130 °C and M-170 °C) flour samples, only one endotherm was observed at a temperature between the starch gelatinisation and protein denaturation peaks. The onset (To) and end (Tc) temperatures for the flours adequately distinguished between the samples, increasing with increase in micronisation temperature. However, the transitional enthalpies (ΔH) did not provide consistent data in relation to the micronisation (41 % moisture, 130 and 170 °C) treatments. There were wide variations in the transitional enthalpies of the flour and isolated starch, which could be attributed to variations due to sample handling (Yu & Christie, 2001) and lack of uniformity in the level of starch granule modification within the (M-130 °C and M-170 °C) seeds. The DSC data for the 130 °C suggests that the sample might have contained some granules that were still in their crystalline state and this supposition was supported by the presence of few birefringent granules in the sample.

Due to the dramatic reduction in paste viscosities of flour from cowpeas micronised to 170 °C as measured using an RVA, it was necessary to investigate if micronisation at higher temperatures had led to depolymerisation of starch. Thermal depolymerisation of starch has been reported in other forms of intense thermal and irradiation treatments (Collona, Leloup & Buléon, 1992; Igura, Hayakawa & Fujio, 1997). Size exclusion high performance liquid chromatography (SE-HPLC) was conducted in order to investigate the possible depolymerisation of starch into smaller water soluble products. SE-HPLC separates molecules according to their molecular sizes (Freifelder, 1982). A stationary phase consisting of a porous matrix is permeated by a mobile phase molecules and the movement of small molecules is impeded in attempting to enter the pores of the matrix while larger molecules are excluded from the matrix and are carried more rapidly through the column (Smith, 1994). Consequently, molecules are eluted in order of decreasing size. However, the method did not yield the expected results, possibly due to dextrinisation and endodegradation of starch which did not yield an increase in water soluble starch products (van den Einde, Goot & Boom 2003; van den Einde *et al*., 2004). Measurement of intrinsic viscosity of the starch would have provided more information as to whether the starch molecules were depolymerised even though the granules were intact. Intrinsic viscosity (η) is a measure of the hydrodynamic volume occupied by a

macromolecule, and is closely related to size and conformation of the macromolecular chains in a particular solvent (Lai & Chiang, 2002). A number of equations are used to derive intrinsic viscosity (η) from the measurement of relative viscosity (η_{rel}) of very low concentration (c) solutions measured using a capillary viscometer (Harding, 1997).

Since the starch granules are embedded in the protein-rich cytoplasmic matrix it was necessary to study the effect of micronisation (41 % moisture, 130 and 170 °C) on cowpea protein as changes in the physicochemical properties of the protein would influence the gelatinisation properties of the starch granules. In addition, some functional properties of cowpea flour such as foaming capacity are dependent on the physicochemical status of the protein. The cowpea protein had to be extracted from the unmicronised and micronised cowpeas (M-130 °C and M-170 °C) to increase the protein concentration and purity of the working sample. Cowpea protein was extracted using isoelectric precipitation technique (Mwasaru *et al*., 1999; Horax *et al*., 2004). Sixty two percent, 20 and 13 % of protein-rich fractions were extracted from unmicronised, M-130 °C and M-170 °C cowpeas, respectively. The method involved the use of a homogeniser (Ultra Turrax T25 -Janke and Kunkel GmbH & Co., K.G., Stauffen, Germany) and 0.1M NaOH to disrupt the integrity of the cytoplasmic matrix and protein body membranes as well as increasing the solubility of the protein. Through the use of isoelectric precipitation and dialysis, relatively pure protein rich fractions with 85, 84, and 62 % protein were extracted from the unmicronised, M-130 °C and M-170 ° C samples, respectively. Reducing the pH of the system to the isoelectric point (pH 4.5) of cowpea protein ensured that the protein precipitated out of solution (Damodaran, 1996a) and could be effectively separated from other soluble components through centrifuging. Dialysis was used to further clean the protein by reducing the concentration of the ions used during the extraction as well as other non-protein components (Freifelder, 1982; Holde, Johnson & Ho, 1998). Despite these extraction and purification steps, the protein–rich fraction yield and purity from the M-170 °C was low possibly due to extensive protein-protein and protein-carbohydrate crosslinking. Since it was difficult to obtain purer protein extract from the micronised seeds (M-130 °C and M-170 °C), micronisation of native protein extract would have provided more information on the possible changes

in physicochemical properties. Under the current experimental design, approximately 80 % of the protein could not be extracted from the micronised seeds (M-130 °C and M-170 °C) and hence could not be studied. Therefore the result obtained on the extracted samples does not fully represent the changes taking place to all of the protein.

SDS-PAGE and fluorescence spectroscopy were used in this study to investigate micronisation-induced physicochemical changes in cowpea protein. SDS-PAGE was used to study the effect of micronisation temperature on the molecular weight and subunit distribution of the cowpea proteins, and formation of disulphide crosslinks. Electrophoresis refers to movement of particles through an electric field and has been used to separate proteins (Freifelder, 1982; Holde *et al*., 1998). Polyacrylamide gels act as molecular sieves and have been used in electrophoresis to separate cowpea proteins according to their molecular weights and subunit composition (Chan & Phillips, 1994; Freitas *et al*., 2004; Horax *et al*., 2004a). To increase the molecular sieving efficacy, 4 - 18 % polyacrylamide concentration gradient gels were used in this study. The gradient gels made by mixing two gel solutions, one with higher concentrated and another one with less concentrated gel material in a gradient mixer were made to give a decreasing porosity downwards in the gel. Gradient gels are preferable for mixed samples with a wide range of molecular size range (Simpson & Whitaker, 1983). Gradient gels produce higher resolution and sharper bands due to in part to the fact that the leading edge of any particular band is moving through more concentrated gel than the trailing edge and hence encounter greater resistance producing a band-sharpening effect (Andrews, 1986). Aluko, Yada, Lencki & Marangoni (1997) used gradient gels (8-25 %) for cowpea globulins resulting in very clear polypeptide band separation. Abu *et al*. (2006) used 7-14 % gradient gels to separate cowpea protein isolate (CPI). The gradient gels prepared in this study successfully separated the cowpea protein both from untreated and micronised seeds (M-130 °C and M-170 °C). This information was critical in providing evidence for the presence of disulphide and other forms of crosslinking in protein extracted from micronised seeds (M-130 °C and M-170 °C).

On the other hand, fluorescence spectroscopy was used to investigate the possible formation of dityrosyl crosslinks and determine changes in the surface hydrophobicity of the proteins. In some molecules, the absorption of a photon is followed by the emission of light of a longer wavelength (i.e. lower energy) known as fluorescence (Freifelder, 1982). Fluorescence measurements in macromolecules such as proteins provide information about conformation, binding sites, solvent interactions, and degree of flexibility, intermolecular distances and the rotational diffusion coefficient of macromolecules. Tyrosine is one of the intrinsic fluors found in proteins and is frequently very weak due to quenching. The unsuccessful measurement of dityrosine in this study could possibly be due to the presence of impurities in the protein-rich fractions used. Tyrosine fluorescence is susceptible to quenching if it is ionised, or near an amino group, a carboxyl group or a tryptophan.

1-anillino-8-naphthalene sulphonate (ANS) was used as an extrinsic fluor to determine the surface hydrophobicity of the protein-rich fractions. ANS fluorescences very weakly in aqueous solution, but in a non polar environment, the quanta increases markedly, shifting the spectrum towards shorter wavelengths (Freifelder, 1982). Extrinsic fluors are used when the study protein does not have natural fluors in the appropriate places of the macromolecule. ANS does not affect the features of the macromolecules under study and it tightly binds to the protein to provide fluorescence in accordance to changes in environmental conditions. Hence ANS has been used to estimate the relative surface hydrophobicity of cowpea protein and globulins (Aluko & Yada, 1993; Mwasaru *et al*., 1999a; Horax *et al*., 2004a) and was successfully used in this study. Despite the shortfall with the purity of the protein-rich fractions, the results followed the expected trend. The surface hydrophobicity of the extracted protein increased with increasing micronisation temperature indicating that more hydrophobic side chains were exposed with increasing micronisation temperature.

4.2 Effect of micronisation (41 % moisture and infrared heating) on physicochemical and structural properties of cowpea seeds, protein and starch

The effect of micronisation in moisture-conditioned seeds was derived from the hydrothermal treatment generated by the internal conversion of electronic energy to vibrational energy and the physical fissuring of the structure due to pressure build in the seed. The proposed mechanisms of changes in seed structure and physicochemical properties of cowpea starch and protein as they affect cooking characteristics of whole seeds and functional properties of the resultant flour are summarised in Figures 4.1 and 4.2.

Therefore, this section will first discuss the effect of micronisation (41 % moisture, 130, 153 and 170 °C) on seed structure, followed by changes in physicochemical properties of protein and starch and their related functional properties in cowpea flour. Later on a thesis will be put forward on how the changes in seed structure and physicochemical properties of protein and starch contributed towards the reduction in cooking time, a softer texture and increased splitting in micronised (41 % moisture, 130, 153 and 170 °C) seeds. Lastly potential applications of flour from moisture-conditioned and micronised cowpea seeds in food systems will be explored.

4.2.1 Effect of micronisation (41 % moisture and infrared heating) on cowpea seed structure

Prior to micronisation, the tempered cowpea seeds were swollen to a certain degree representing an initial increase in volume. During micronisation (41 % moisture, 130, 153 and 170 °C), the increased molecular vibrations of water led to rapid increase in temperature and vaporisation of water molecules, further increasing the volume and pressure within the seed (Fasina *et al*., 2001). The reduction in bulk density of the moisture-conditioned and micronised seeds was evidence of the increase in volume (Table 4.1), although the high moisture content of the M-130 °C samples masked this effect. The increase in pressure resulted in visible cracks on the seed coat, as well as microscopic fissuring of the seed coat, cotyledon and cell walls.

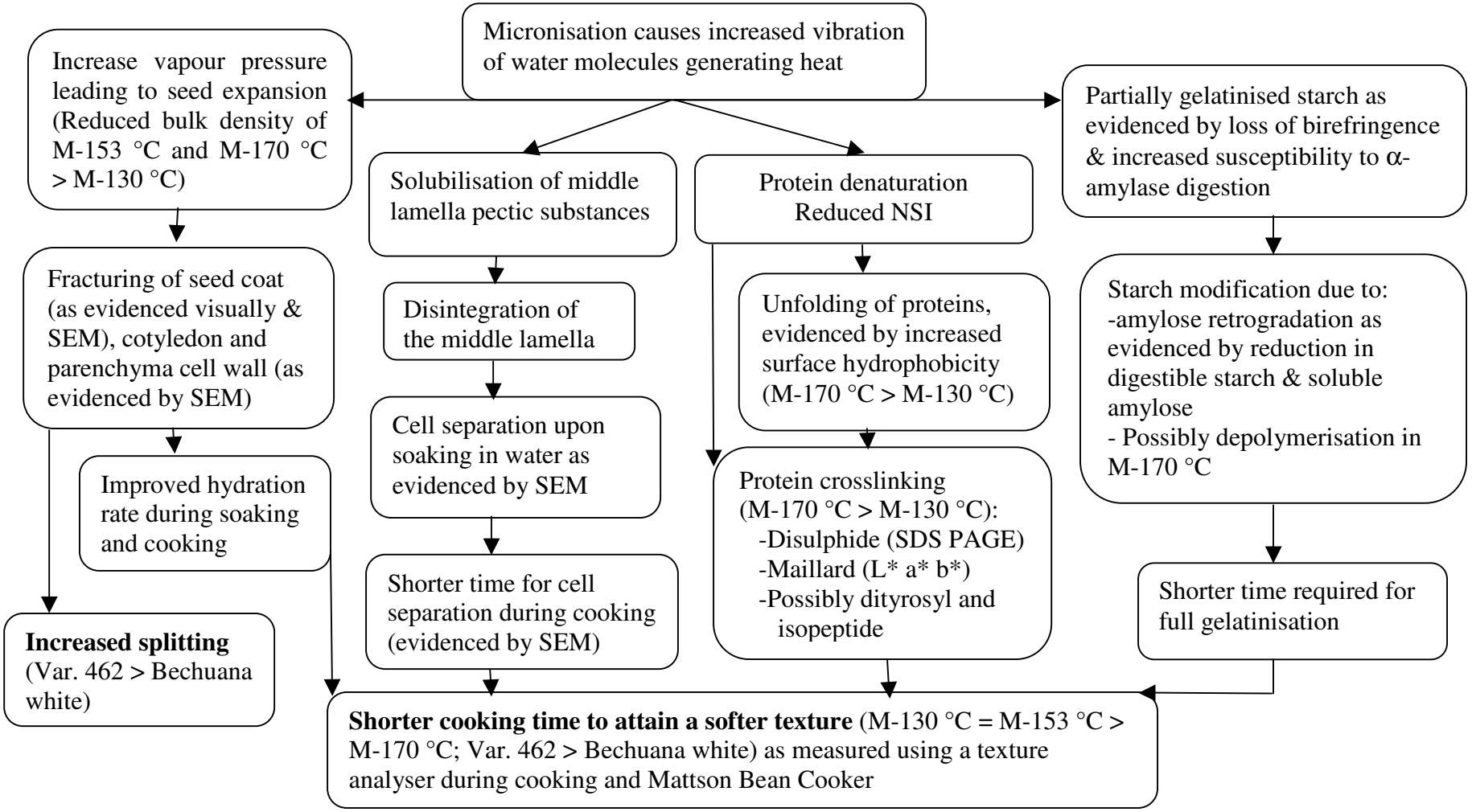

Figure 4.1 **Proposed mechanisms of the effect of micronisation (41 % moisture, 130, 153 and 170 °C) on cowpea seed structure and the physicochemical properties as related to cooking characteristics of cowpea seeds**

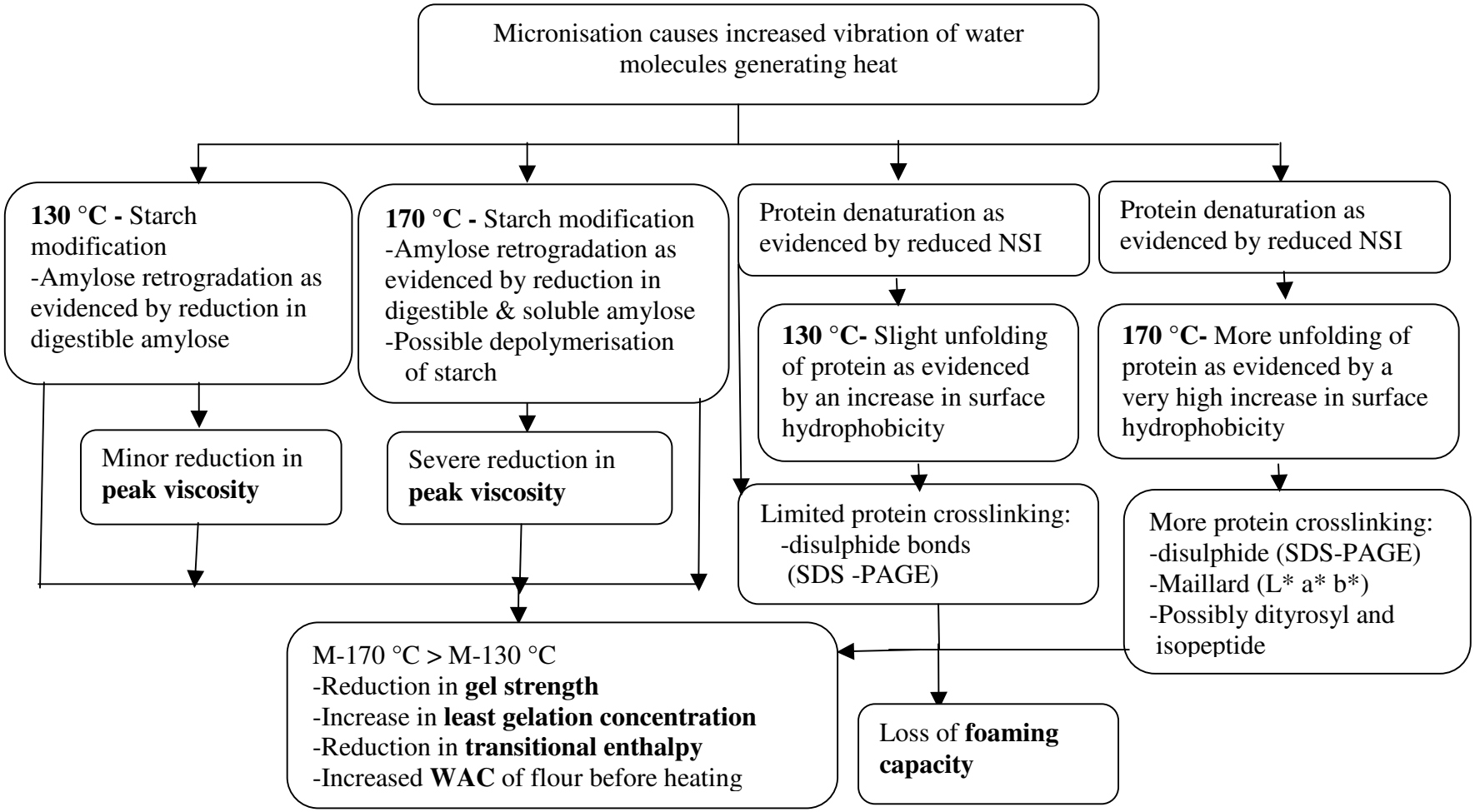

Figure 4.2 **Postulated effect of cowpea seed micronisation (41 % moisture, 130 and 170 °C) on physicochemical properties of starch and protein and functional properties of flour milled from the micronised (41 % moisture, 130 and 170 °C) seeds', NSI= Nitrogen solubility index, WAC= Water absorption capacity**

Table 4.1 Summary of changes in physicochemical properties of cowpea seeds, flour and protein fraction following micronisation to different temperatures in relation to unmicronised samples

Physicochemical property		Percentage change (%) in relation to unmicronised samples*			
		Var. 462	Bechuana white		
		153 °C	130 °C	153 °C	170 °C
Bulk density		↓ 20	→	↓24	↓29
Hydration capacity		↓27	↓27	↓21	↓12
Cooking time		↓36	↓ 47	↓44	↓ 32
Water absorbed	30 min of cooking	↑53	↑36	↑59	↑699
	60 min of cooking	↑9	↓8	↑11	↑8
Texture	30 min of cooking	↓42	↓36	↓32	↓36
	60 min of cooking	↓32	→	↓19	→
Splitting	30 min of cooking	↑∞	↑1233	↑1366	↑1300
	60 min of cooking	↑1464	↑64	↑ 38	→
Enzyme susceptible starch		↑509	↑ 248	↑ 223	↑ 223
Amylose (digestible)		ND	↓23	ND	↓24
Flour thermal properties					
On set temperature		ND	↑6	ND	↑6
Transitional enthalpy		ND	↑58	ND	↓27
L*		ND	↓3.6	ND	↓17
a*		ND	↑4.6	ND	↑69
b*		ND	↑4.5	ND	↑121.8
Nitrogen solubility index		↓61	↓32	↓61	↓46
Surface hydrophobicity		ND	↑62	ND	↑174

*↑ = Significant increase, →= No change, ↓ = Significant decrease; ∞= divisible with zero since the unmicronised samples had zero splits; ND= Not determined

The fissures occurred during micronisation (41 % moisture, 130, 153 and 170 °C) of all the cowpea samples (130, 153, 173 °C) and in both varieties.These fissures probably contributed to the increased rate of water absorption during cooking as well as splitting of cooked seeds (Table 4.1). The presence of fissures in micronised legumes was alluded to by previous researchers (Arntfield *et al*., 2001) but was clearly demonstrated in this work. This work has clearly shown that micronisation (41 % moisture, 130, 153 and 170 °C) resulted in visible cracking of the seed coat, as well as microscopic fissuring of the seed coat, on the cotyledon surface and cross section as well as on the cotyledon parenchyma cell walls.

4.2.2 Effect of micronisation (41 % moisture and infrared heating) on physicochemical properties of cowpea protein and protein-related functional properties of the resultant flour

It is proposed that due to the hydrothermal effect of micronisation, denaturation of cowpea protein was induced through the breakage of intra and intermolecular hydrogen bonds leading to the unfolding of the protein molecules to expose previously buried hydrophobic sites as evidenced by the significant increase in surface hydrophobicity of the proteins (Table 4.1). The unfolded proteins rearranged with the formation of covalent bonds. These covalent bonds included disulphide bonds, as indicated by SDS-PAGE gels of the protein-rich fraction isolated from raw and micronised (41 % moisture, 130 and 170 °C) seeds. Other possible crosslinks include isopeptide (glutamyl-lys) and dityrosyl bonds as well as crosslinks derived from Maillard reactions. Isopeptide crosslinks would possibly be involved in samples micronised to 170 °C since very high temperatures were used (Gerrard, 2002) and SDS-PAGE showed new proteins bands that were not reduced by mercaptoethanol.

Cowpea protein contains lysine (Chan & Phillips, 1994) and would be involved in Maillard reactions in the presence of reducing sugars (Ukhun, 1987) producing water insoluble browning polymers known as melanoidins (Rizzi, 1994; BeMiller & Whistler, 1996). It has been indicated that dicarbonyl compounds derived during the Maillard

reaction, such as methylgloxal or 3-deoxyosones attach to lysine, arginine, and tryptophan residues of the protein via one of their bifunctional groups. The complexed proteins polymerise through binding of the second functional group (carbonyl) with remaining lysine and arginine residues of the protein (Oliver, Melton & Stanley, 2006).

In the present study, the occurrence of Maillard reactions was assessed by measuring the lightness (L*), redness (a*) and yellowness (b*) of the flour, since Maillard reactions generate products with brown and yellow coloured pigments (Whistler & Daniel, 1985). The results as summarised in Table 4.1 indicated that more of the brown pigmentation was formed in the cowpeas micronised to 170 °C than the samples micronised to 130 °C. Hence it was construed that more Maillard reactions occurred in the M-170 °C samples than in the M-130 °C cowpeas. Arntfield *et al*. (2001) and Phadi (2004) suggested that the browning observed in moisture-conditioned and micronised lentils and cowpeas at higher temperature (>160 °C) was possibly due to Maillard browning. Measurement of available lysine would have ascertained the occurrence of Maillard reactions in the moisture-conditioned and micronised samples. Lysine concentration is usually measured as an indicator of Maillard reactions due to the epsilon amino group which readily participates in Maillard reactions (Assoumani, Maxime, Nguyen, 1994; BeMiller & Whistler, 1996; Damodaran, 1996a). Thus Maillard reactions may lead to reduction in the bioavailability of lysine (Damodaran, 1996a). Maillard browning may not be the only possible cause of browning in flour from moisture-conditioned and micronised cowpea seeds. Abu *et al*. (2006a) reported that the browning of irradiated cowpea flour was not accompanied by significant decrease in available lysine. Other possible causes of browning could be polymerisation of oxidised phenolics (Onigbinde & Onobun, 1993) and formation of melanin-type pigments from the oxidation of tyrosine and tryptophan as reported in irradiation treatments (Ley, Bleby, Coates & Petterson, 1969; Diehl, 1990).

Dityrosyl crosslinks are another possible form of crosslinks that could result in protein polymerisation during micronisation (Fig 4.1). Dityrosine is a covalently bonded biphenol produced by reaction of two tyrosyl radicals or a tyrosyl radical plus a tyrosine molecule. Dityrosyl crosslinks occur at intramolecular and intermolecular (Kanwar &

Balasubramanian, 1999) levels, with the latter leading to protein polymerisation (Davies, 1987). Damodaran (1996a) indicated that reactive unsaturated carbonyls and free radicals formed during Maillard browning reaction could cause oxidation of amino acids such as tyrosine thus facilitating the formation of dityrosyl crosslinks in the protein. In the present study, measurement of the formation of dityrosine in protein extracted from moisture-conditioned and micronised cowpeas was unsuccessful due to sample impurity.

The increase in surface hydrophobicity of the cowpea protein due to molecular unfolding and formation of crosslinks led to the denaturation of the protein which was evident by significant reduction in nitrogen solubility index in both cowpea varieties at all micronisation temperatures. However, the maximum reduction in NSI was observed in the M-153 °C (Table 4.1). The slight improvement in soluble nitrogen in the M-170 °C despite the high surface hydrophobicity and crosslinking suggest the possibility of protein thermal degradation. Finley (1989) indicated that when proteins are exposed to temperatures ranging from 150 - 200 °C covalent bonds may be broken resulting in peptidisation. However, the SDS-PAGE of the protein extract from moisture-conditioned cowpeas micronised to 170 °C did not show an increase in lower molecular weight peptides. These could have been lost during the protein isolation process which included dialysis using dialysis tubes with 12-14 kDa pore size.

It has been reported that most of the protein-related functional properties are highly dependent on protein solubility (Damodaran 1996b). The reduction in NSI of the cowpea flour was accompanied by significant reduction in foaming capacity and water solubility index (Table 4.2). The flours from micronised (M-130 °C and M-170 °C) cowpea seeds lost their ability to foam. The loss in foaming capacity was possibly due to protein crosslinking, especially disulphide bonds which probably reduced the molecular flexibility of the protein. Aluko and Yada (1999) reported that formation of disulphide bonds resulted in reduced molecular flexibility, which is crucial for foam formation.

The reduction in water solubility index of the flour from micronised (M-130 °C and M-170 °C) seeds was mainly due to protein denaturation. Water solubility index

measures the presence of water soluble compounds in flour which include water soluble protein and low molecular weight carbohydrates. Micronisation (M-130 °C and M-170 °C) reduced the protein solubility of the flours, but there was no change in the level of low molecular weight carbohydrates (M-170 °C).

Micronisation (M-130 °C and M-170 °C) significantly increased the water absorption capacity (WAC) of the flours (Table 4.2). Heat treatment has been reported to increase water absorption capacity of flour made from micronised legumes (Fasina *et al*., 2001) and cereals (Fasina *et al*., 1999).

Table 4.2 Summary of percentage change (%) in functional properties of cowpea flour from cowpea seeds micronised to 130 and 170 °C in relation to unmicronised samples

Functional property	Micronisation (°C)*	
	130	170
Water absorption capacity (WAC)	↑81	↑107
Oil absorption capacity (OAC)	→	→
Water solubility index (WSI)	↓16	↓21
Swelling index (SI)	↓18	↓18
Least gelation concentration (LGC)	↑38	↑63
Gel strength (GS)	↓38	↓75
Foaming capacity (FC)	↓62	↓65
Cowpea flour pasting properties		
Peak viscosity	↓15	↓79
Break down	↓91	↓94
Set back	↓32	↓60

*↑ = Increase, →= No change, ↓ = Decrease

Water absorption capacity represents the flour's ability to associate with water and is required in products such as pastes and doughs. Phillips *et al.* (1988) observed that water holding capacity of cowpea flour increased with mild heat pre-treatment of the seeds and declined with severe heat pre-treatment. It is apparent that water absorption capacity of the flour from micronised seeds (M-130 °C and M-170 °C) was not dependent on protein solubility. While micronisation (41 % moisture, 130 and 170 °C) induced the unfolding of proteins as evidenced by increase in surface hydrophobicity, more hydrophilic sites that were buried in the native protein were also made available to associate with water. Hence this would explain the higher water absorption capacity of M-170 °C as compared to M-130 °C.

4.2.3 Effect of micronisation (41 % moisture and infrared heating) on physicochemical properties of cowpea starch and starch-related functional properties of the resultant flour

Micronisation of moisture-conditioned cowpeas caused disruption of starch granular order resulting in the loss of birefringence and increased susceptibility to α-amylase digestion which was observed in all micronised samples (M-130, M-153 and M-170 °C). However, the increase in starch enzyme susceptibility was higher in Var. 462 than Bechuana white at M-153 °C (Table 4.1). Since prior to micronisation (41 % moisture, 130, 153 and 170 °C) Bechuana white cowpeas had higher enzyme susceptible starch than Var. 462, it implies that micronisation (41 % moisture, 153 °C) affected Var. 462 starch granular order more than Bechuana white. This could possibly be due to differences in seed and starch granule structure.

Due to the disruption of the native starch granular order, the amylose molecules were inclined to re-associate and retrograde within the starch granules. This retrogradation of amylose was evidenced by reduction in digestible amylose in both M-130 °C and M-170 °C. Thermal properties of the starch extracted from the unmicronised and micronised samples (M-130 °C and M-170 °C) showed that there was a reduction in crystallinity of the granules. However, the pasting properties of starch extracted from micronised seeds (M-130 °C and M-170 °C) were not a true reflection of the pasting

characteristics of starch in the seeds due to very low extraction rates. Nevertheless, pasting properties of the flour showed the absence of cold swelling and reduced pasting viscosity in the order of increasing micronisation temperature (Table 4.2).

The absence of a cold swelling system was attributed to the presence of intact cells which trapped the starch granules that had been modified following micronisation (41 % moisture, 130 and 170 °C) as observed by light microscopy in this study. Another reason for the absence of the cold swelling system could possibly be a rigid starch granule structure as a result of re-association of amylose molecules within the granule. Similar absence of cold swelling systems has been observed in the work reported by Cenkowski & Sosulski (1998) on micronised peas. Lai (2001) also observed the absence of a cold swelling system in pregelatinised rice flour from two rice varieties which was attributed to amylose re-association.

The decline in pasting properties may be ascribed to amylose re-associations both in the moisture-conditioned cowpeas micronised to130 °C and 170 °C and possibly depolymerisation of both amylose and amylopectin in moisture-conditioned cowpeas micronised to 170 °C (Figures 4.1 and 4.2). The slight increase in gelatinisation onset temperature (To) and decline in the peak viscosity of the M-130 °C (Table 4.2) showed that although the starch granules in the M-130 °C cowpeas contained some retrograded amylose, the granules retained their ability to absorb water and swell during gelatinisation. This result was in contradiction to the increased pasting viscosity that was observed by Cenkowski & Sosulski (1998) for flour from micronised peas (26 % moisture, 120 °C). The increase in pasting viscosity was attributed to the presence of free granules in the flour. However, light microscopy of the M-130 °C cowpea flour in this study showed more intact cells than free starch granules. Another possible reason for the difference in the pasting viscosity would be the level of pregelatinisation, since the moisture content and temperatures used in the study by Cenkowski & Sosulski (1998) were lower than what was used in this study.

The starch granules in the M-170 °C exhibited very limited swelling, which was attributed to retrograded amylose, space restriction due to protein crosslinking within the intact cells and possibly depolymerisation of starch polymers. Although there was no significant increase in water soluble starch products due to micronisation (41 % moisture, 170 °C) as determined by HPLC-GP, it was evident from the starch extraction data that the starch could not be easily extracted from the flour. Furthermore, depolymerisation of starch has been reported during other physical treatments such as intense heating (20 % moisture, 150 °C) in the absence of shear (Igura *et al*., 1997) and γ-irradiation (Rombo, Taylor & Minnaar, 2004). It has been reported that γ-irradiation (10 kGy) of starch produced similar effects as thermal (125 °C for 1 h) treatment of starch (Raffi, Agnel, Frejaville & Sait-Lebè, 1981). Exodegradation of heated potato, maize and wheat starch as evidenced by increased cold water solubility, and reduction in molecular size (gel permeation filtration) has been reported (Igura *et al*., 1997). At the same time, intense heating (100-160 °C) of maize starch (43 % moisture) in the absence of shear resulted in decreased intrinsic viscosity, indicating the endodegradation of starch (van den Einde *et al*., 2004). Micronisation, especially at the higher temperatures could therefore possibly result in the depolymerisation or dextrinisation of cowpea starch.

The change in physicochemical and pasting properties of the starch affected starch-related functional properties of the flour, namely gelling and swelling. Gelation in a heterogeneous system such as cowpea flour is attributed to protein and starch functionality (Prinyawiwatkul *et al*., 1997a). The gelation properties of these macromolecules would affect several functional properties of the cowpea flour which include gel strength and least gelation concentration of the flour. In the present study there was significant reduction in gel strength and increased concentration at gelation for micronised (M-130 °C and M-170 °C) flour. Starch contributes to gelation through association of dispersed amylose as exhibited by set back properties of unmicronised flour. Since micronisation (41 % moisture, 130 and 170 °C) resulted in retrogradation of amylose (reduction in digestible amylose, Table 4.1) within the starch granules, it implies that the dispersed amylose during subsequent pasting of the flour was reduced hence contributing to a weaker gel as well as increasing the least gelation concentration. The

reduction in gel strength was greater in flour from M-170 °C than in the flour from M-130 °C cowpeas (Table 4.2). The possible depolymerisation of the starch contributed to further weakening of the gel formed by the M-170 °C cowpea flour since there was no significant difference in the levels of digestible amylose between the M-130 °C and M-170 °C cowpea flour. Starch depolymerisation was reported to contribute towards the reduction in gel strength of starch isolated from γ-irradiated cowpea flour (Abu *et al*., 2006b). Irradiation effects on starch have been reported to be similar to heat treatment (Raffi *et al*., 1981). Furthermore the formation of more disulphide bonds in the M-170 °C could contribute towards the reduction in protein gelation since disulphide bonds are reported to reduce molecular flexibility and consequently gel network formation (Phillips, Whitehead & Kinsella, 1994). The flour from the micronised seeds (M-130 °C) however retained relatively good gelling properties that could be utilised in food systems.

4.3 Proposed mechanism of micronisation-induced (41 % moisture and infrared heating) changes in cooking characteristics of cowpea seeds

As mentioned earlier in section 4.1, cooking time is of primary importance among the different cooking characteristics of dried cowpea seeds. Cooking time of dried cowpea seeds is derived from the time required to soften the seed texture during cooking. The softening of seed texture during cooking has mainly been explained in terms of physical and chemical changes that take place in the cotyledon (Sefa-Dedeh & Stanley, 1979a; Liu *et al*., 1993a). Figure 4.3 presents a diagrammatic presentation of the cotyledon parenchyma cells, protein and starch in cowpea seeds and the postulated scheme for the softening of cowpea cotyledon during cooking of untreated seeds.

This section of the discussion will now attempt to explain the mechanism by which micronisation (41 % moisture, 130, 153 and 170 °C) reduced the cooking time of cowpeas, and increased the tendency of splitting as illustrated in Figure 4.4. Focus will be placed on changes in cowpea seed structure and physicochemical properties of protein and starch. First, the mechanism involved in cooking of untreated cowpea seeds will be

put forward, and will form the basis for the discussion of the effect of micronisation (41 % moisture, 130, 153 and 170 °C) on cooking characteristics.

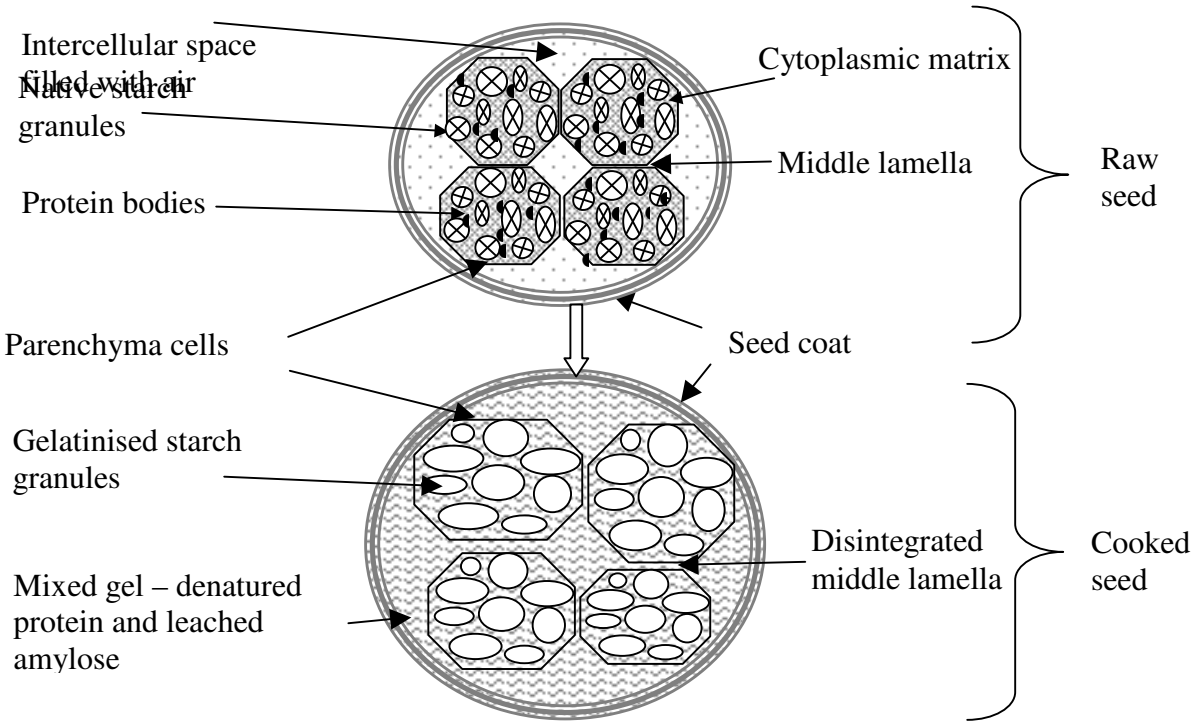

Figure 4.3 **Diagram showing the suggested changes in cowpea structure at the microscopical level that lead to softening of texture during cooking of unmicronised (raw) cowpeas** (Based on the mechanism proposed by Liu *et al*., 1992; Liu *et al*., 1993a; and Liu *et al*., 1993b)

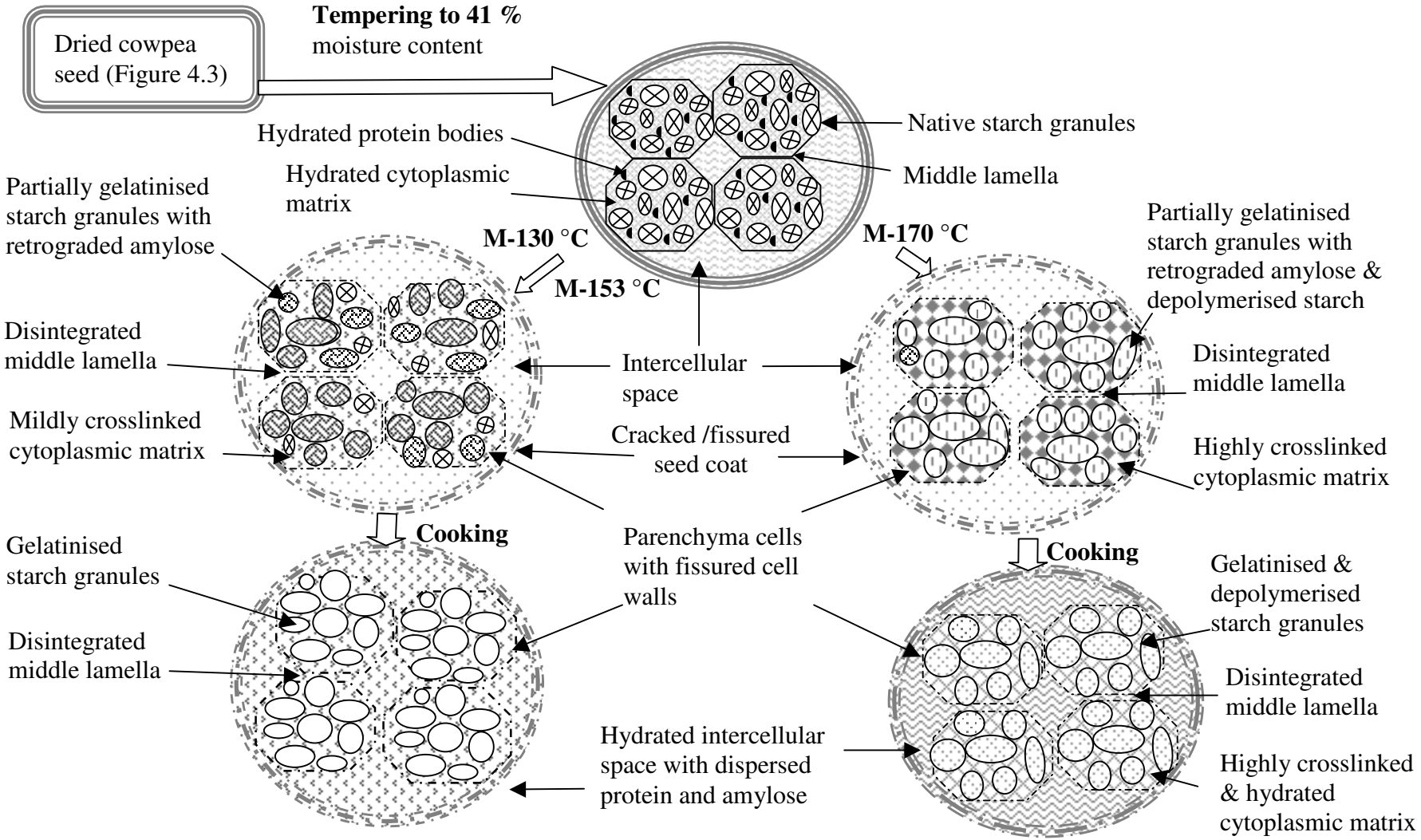

Figure 4.4 **Diagram showing the suggested changes in cowpea structure that lead to softening of texture during cooking of micronised (41 % moisture, 130, 153 and 170 °C) cowpeas**

The middle lamella holds the individual parenchyma cells together, hence conferring a fixed structure to the cotyledon. The middle lamella consists of pectic substances, which are heat sensitive at near neutral pH (Liu *et al*., 1993b). In the present study, SEM of 60 min cooked cowpea samples (Var. 462 and Bechuana white) showed parenchyma cell separation confirming what has been reported by earlier researchers that when cowpea seeds are heated at temperatures above 85 °C in the presence of water, the pectins in the middle lamella degrade to lower molecular weight products through the β-elimination reaction (Liu *et al*., 1993b). With the middle lamella degraded, the glue that holds the individual parenchyma cells in place is reduced, thereby contributing to a soft texture.

In the native state, starch granules exist as granules with crystalline structure and are in a glassy state conferred by the amorphous area (BeMiller & Whistler, 1996). During cooking in water, starch granules absorb water which acts as a plasticizer leading to phase transition from a glassy to a rubbery state (Parker & Ring, 2001). A glass is a mechanical solid capable of supporting its own weight against flow. A rubber is an under cooled liquid that can exhibit viscous flow (BeMiller & Whistler, 1996). Fisher, Carrington & Odell (1997) used potato starch to demonstrate that swollen starch granules behave as reversible and highly deformable bodies. With such mechanical properties, gelatinised granules in the cowpea cotyledon contribute towards the softening of the cowpea texture. However, this is dependent on the state of the protein-rich cytoplasmic matrix which envelopes the starch granules.

It has been proposed that protein contributes to the softening of the cowpea cotyledon through its effect on starch gelatinisation (Liu *et al*., 1993a; Liu *et al*., 1993b). During cooking, heat-induced unfolding and aggregation or gelation could take place, all of which depends on solubility and thermal stability of the protein (Liu *et al*., 1992). In native cowpea seeds, water extractable protein constitutes about 76 % of the total protein and has a higher thermal denaturation temperature than the starch (Liu *et al*., 1992). Due to this property, denaturation of cotyledon protein usually occurs after starch gelatinisation. With the gelatinisation of starch within the whole seed, amylose leaches from the granules (BeMiller & Whistler, 1996) to the cytoplasmic matrix. Some of the

leached amylose would move out of the cell into the intercellular space together with protein and further out of the seed coat. Release of cowpea protein from cowpea seeds into cooking water has been reported by Hirano, Kagawa and Okubo (1992). Through the use of ESEM coupled with either protease or pectinase digestion, this study has shown that there were pectic substances and protein material outside the cotyledon parenchyma cells of cooked cowpeas. Therefore during cooking of native cowpea seeds, a composite gel consisting of protein and amylose was formed within and outside the parenchyma cells. The mixed gel thus formed, enhanced the water holding properties of the cooked seeds hence increasing the plasticizing effect of water and contributing to softness. This idea is supported by the highly significant negative correlation that was observed in this study between water absorption during cooking and texture of the cowpeas. In addition, Bechuana white had higher water absorption during cooking than Var. 462 and it followed that it was softer following subsequent cooking.

It is proposed that in moisture-conditioned and micronised cowpeas, the splitting observed during the initial 30 min of cooking could be ascribed to the micronisation-induced fissuring. However after the initial 30 min of cooking the normal mechanism for splitting would be responsible. This suggestion is well illustrated by the splitting pattern observed in micronised Var. 462 (M-153 °C) cowpeas and the M-170 °C Bechuana white cowpeas. It is proposed that splitting during cooking of untreated seeds is a factor of seed density, and the interplay between starch and protein during cooking. Taiwo *et al*. (1998) reported that splitting of cooked cowpeas correlated positively with drained weight and softness (penetration depth). Since starch and protein are the main water absorbing and holding entities during cooking of cowpea seeds, it means that starch gelatinisation behaviour and protein denaturation would help to explain the splitting phenomena. Gelatinisation characteristics of starch are dependent on its amylose/amylopectin ratios. It has been shown that swelling power of starch granules decreases with increasing amylose content (Czuchajowska, Otto, Paszczynska & Baik, 1998; Zheng, Han & Bhatty, 1998). This would imply that cowpea seeds with higher amylopectin content would have a higher propensity to absorb water and swell more during cooking, requiring additional seed volume for expansion (There was no significant difference in amylose content of the

two cowpea varieties used in this study; results for Var. 462 were not shown). This would not be a problem in cowpeas with low density, like Var. 462, but in seeds of high density coupled with a thin seed coat as was the case for Bechuana white, space for expansion would be limited, leading to splitting. This would explain why unmicronised Var. 462 had minimal splitting during cooking and there was no significant increase in splitting during extended cooking of micronised Var. 462 (M-153 °C) since the splitting during the first 30 min was attributed to micronisation-induced fissuring. In addition to cotyledon characteristics, the incidence of splitting has been negatively correlated with calcium, magnesium and sodium content of the seed coat in kidney bean (Wu, James & Anderson, 2005).

As mentioned earlier, interplay between the behaviour of starch granules and that of the protein-rich cytoplasmic matrix also contributes to splitting of seeds during cooking. Assuming that micronisation-induced fissuring was responsible for the splitting observed during the initial 30 min of cooking, then it would suggest that the lack of increase in splitting for the M-170 °C during extended cooking (45-90 min) was due to changes in the behaviour of starch granules and the protein matrix. Although the water absorption pattern for the M-170 °C during cooking was higher than the other samples (Table 4.1), the M-170 °C did not have higher incidence of splitting during extended cooking (45-90 min). Therefore examination of starch and the protein (cytoplasmic matrix) behaviour would assist in explaining the splitting as well as the shorter cooking time of moisture-conditioned and micronised seeds.

Based on the level of crosslinking in protein extracted from the moisture-conditioned and micronised seeds, it is proposed that the denatured cowpea protein network formed in moisture-conditioned cowpeas micronised to mild temperatures (130 °C and 153 °C) was different from the one formed in moisture-conditioned cowpeas micronised to 170 °C (Figure 4.4). Although the protein in micronised seeds (130 °C, 153 °C and 170 °C) was denatured and had high surface hydrophobicity, the denatured protein network was able to absorb and hold capillary water which is loosely bound (Fennema & Tannenbaum, 1996; Damodaran, 1996b). With the increase in temperature during cooking, the

capillary water was available for gelatinisation of starch granules to progress during subsequent cooking. Since the denatured protein network formed in the 130 °C might have been weaker, due to the formation of fewer crosslinks, it was flexible and allowed the starch granules to swell and acquire a rubbery state thus contributing to a softer texture, shorter cooking time and continued splitting during extended cooking (Table 4.1).

However, in moisture-conditioned cowpeas micronised to 170 °C, the denatured protein network formed was possibly stronger due to the presence of more crosslinks. Mitchell & Hill (1995) reported that protein gels formed in the process of Maillard reactions have higher gel strength than the gels formed in the absence of Maillard reactions. Although the M-170 °C seeds were capable of absorbing more water (Table 4.1), the starch granules could not swell due to space restriction and possibly depolymerisation of the starch polymers (Figure 4.4). Due to these changes in the protein matrix and the starch, moisture-conditioned cowpeas micronised to 170 °C had a slightly longer cooking time than the M-130 °C and the seeds did not continue to split during extended cooking.

In addition to the changes in starch and protein, micronisation (41 % moisture, 130, 153 and 170 °C) treatment resulted in the degradation of middle lamella as evidenced by separation of parenchyma cells in moisture-conditioned and micronised seeds when soaked in water as well as in 30 min cooked micronised seeds. From the observed separation of cells using SEM it is deduced that micronisation (41 % moisture, 153 °C) might have induced beta elimination of pectic substances which contributed to shorter time requirement for cell separation during cooking (Figures 4.3, 4.4). This hypothesis will have to be tested, by determining the changes in pectic substances following micronisation of moisture-conditioned seeds. Hydrothermally-induced degradation of the middle lamella has been reported in moisture-conditioned and micronised lentils (Arntfield *et al*., 2001) as well as hot air treated cowpeas (25 % moisture; 110-130 °C) (Hung *et al*., 1990).

4.4 Potential uses of cowpea flour from moisture-conditioned and micronised seeds

Micronisation of moisture-conditioned cowpeas seeds resulted in a loss in foaming properties, reduction in gelling properties and water solubility index as well as increase in water absorption capacity in the resultant flour. Despite the loss of the foaming property, cowpea flour milled from moisture-conditioned and micronised seeds could still be used in other food systems as indicated in Table 4.3. The manufacturing of some processed foods require ingredients that gel during thermal processing to provide a structural matrix for holding water and other ingredients while also providing desirable texture and mouthfeel (Phillips *et al*., 1994).

Since cowpea flour from moisture-conditioned and micronised seeds retained a level of gelation properties, the flours could form gels and provide a structural matrix for holding water, flavours, sugars and other food ingredients. This is a desirable property that would be useful in food applications and new product development.

Due to the reduction in soluble proteins, cowpea flour from moisture-conditioned and micronised seeds could be incorporated into many bakery products at moderately high levels, because it would not interfere with gluten formation. However, it has been reported that incorporation of extruded cowpea flour (30 %) during bread making affected loaf volume more than incorporation of native flour (McWatters *et al*., 2005). Incorporation of raw cowpea flour in bakery products such as cookies and bread has been associated with a raw legume flavour and hardness of texture (McWatters *et al*., 2003; Hallén *et al*., 2004; McWatters *et al*., 2005). It has been suggested that a form of hydrothermal pre-treatment such as extrusion and micronisation would be necessary to reduce the raw legume flavour and the level of functional protein that contributes to a harder texture (McWatters *et al*., 2003; Hallén *et al*., 2004).

Flour from moisture-conditioned and micronised cowpeas could also be used to blend with cereals in complementary foods for children. Traditionally, cereals and legumes are

roasted to reduce cooking time, deactivate anti-nutritional factors and develop aroma. Griffith & Castell-Perez (1998) reported that roasting (25 min, 11.9 % moisture and 145 °C) of cowpeas did not affect the viscosity of gruels prepared from the flours. Similarly the mild micronisation treatment (130 °C) mildly reduced the pasting properties of cowpea flour. However, the M-170 °C micronisation treatment significantly reduced the peak viscosity of the flour and caused considerable browning and would possibly have a burnt aroma. The reduced viscosity of the flour could allow for higher levels of cowpea incorporation in weaning foods hence improved protein content. However, there is need to conduct digestibility studies for the moisture-conditioned and micronised cowpeas to assess the possible negative effect of Maillard reactions and starch retrogradation. Although micronisation of moisture-conditioned seeds has been reported to improve the digestibility of legumes relative to uncooked ones when used in animal feed (Pickard, 1999), it is yet to be elucidated if this is so when compared to normally cooked products. Maillard reactions are associated with reduced availability of lysine while retrograded starch is resistant to enzyme digestion. These properties may impact negatively on the utilisation of cowpea flour from moisture-conditioned and micronised seeds in complementary foods for children.

5 CONCLUSIONS AND RECOMMENDATIONS

Micronisation (41 % moisture and infrared heating) reduces the cooking time and increases the incidence of splitting in cowpeas during subsequent cooking. The effect of micronisation (41 % moisture and infrared heating) on cooking characteristics of the cowpeas differs based on variety and micronisation temperature. Micronisation of moisture-conditioned cowpea seeds to higher temperatures adversely affects functional properties of the resultant flour.

The effect of micronisation (41 % moisture, 153 °C) on cooking characteristics (cooking time, texture and extent of splitting) of two cowpea varieties is not identical. The difference in the reaction of the varieties to micronisation (41 % moisture, 153 °C) is a result of differences in compactness of the cotyledon structure and starch properties such as enzyme susceptible starch.

The reduction in cooking time as a result of micronisation (41 % moisture, 130, 153 and 170 °C) is attributed to development of fissures, starch modification and protein denaturation. Micronisation (41 % moisture, 130, 153 and 170 °C) produces fissures in the cowpea seed coats and cotyledons, which leads to improvement in water uptake, a softer texture and increased splitting during cooking. Therefore, it is recommended that cowpeas that are susceptible to splitting during cooking should not be used for micronisation processing if whole cowpeas are desired after cooking. However, cowpeas susceptible to splitting can be micronised for processing into products such as split soups and flour. Based on the results observed in splitting of unmicronised and micronised (41 % moisture, 130, 153 and 170 °C) cowpeas, this work has elaborated the mechanism involved in splitting of cowpeas during cooking. This mechanism involves seed density and the interplay of starch granules and the protein matrix.

The starch in moisture-conditioned and micronised cowpea seeds is partially gelatinised which leads to the modification of the starch granular structure probably through amylose

associations as evidenced by reduction in digestible amylose and pasting viscosities. However, under mild micronisation conditions (130 and 153 °C) the starch granules retain the ability to undergo the gelatinisation process. At high (170 °C) micronisation temperature amylose associations are possibly accompanied with endodegradation of starch polymers, which leads to substantive reduction in pasting properties. Simultaneously, micronisation of moisture-conditioned cowpeas induces the unfolding of protein exposing more hydrophobic side chains hence increasing the surface hydrophobicity of the proteins. The unfolded proteins form crosslinks especially at higher micronisation temperature (M-170 °C) as a result of Maillard reactions as well as disulphide and possibly dityrosyl and isopeptide bonds. The increased protein crosslinking in moisture-conditioned cowpeas micronised to higher temperatures (170 °C) and possible depolymerisation of starch, contributes to hardening of the cotyledon texture resulting in longer cooking time and reduced splitting during extended cooking time.

The changes in physicochemical properties of cowpea proteins severely affect the functionality of cowpea protein resulting in the loss of foaming capacity. The flours from moisture-conditioned and micronised seeds have high water absorption capacity and good gelling properties especially for the mild treatment (130 °C). However, flours from moisture-conditioned cowpeas micronised to high temperatures (170 °C) have limited application in food systems due to the decline in most of the functional properties measured in this study.

It is recommended that micronisation of isolated cowpea protein and starch be conducted in order to elucidate the extent of modification in physicochemical and functional properties of these components, since this was not fully explored due to interactions with each other and other components in a flour system. In addition, intrinsic viscosity of the starch will have to be measured to verify the endodegradation of starch polymers.

The reduction in digestible amylose in moisture-conditioned and micronised seeds could signal the possible increase in resistant starch, while the denaturation of the protein-rich cytoplasmic matrix could contribute towards an increase in slowly digestible starch.

These factors would contribute to a reduction in the already low glyceamic index of cowpeas. Hence it is recommended that in-vitro starch digestibility studies of moisture-conditioned and micronised cowpeas be conducted to ascertain these possible benefits.

Since this study has shown that micronisation of moisture-conditioned seeds can be used successfully to reduce the cooking time of cowpeas, it is recommended that sensory studies be conducted to determine the effect of micronisation (moisture conditioning and infrared heating) on sensory characteristics and possible consumer acceptance of the moisture-conditioned and micronised whole cowpeas and flour-based products. The success of micronisation (moisture conditioning and infrared heating) as a pre-treatment for cowpeas would depend on consumer acceptance of the end products.

This study has shown that micronisation (moisture conditioning and infrared heating) of cowpea seeds to moderate temperatures through its effect on the physical structure and modification of starch and protein can be used as a pre-treatment of cowpea seeds to produce cowpea seeds with shorter cooking time and cowpea flour with modified functionality. As such micronisation of moisture-conditioned seeds could be one of the alternative processes for diversifying the utilisation for cowpea seeds.