1 INTRODUCTION

Cowpeas are leguminous seeds that are widely produced in Africa under marginal production systems. Cowpeas perform well even when produced in marginal soils due to their ability to fix substantial nitrogen in the soil (Hall, Cisse, Thiaw, Elawad, Ehlers, Ismail, Fery, Roberts, Kitch, Murdock, Boukar, Phillips & McWatters, 2003). In Malawi, cowpeas are produced country wide as an intercrop with maize particularly in warm areas with low rainfall such as Shire valley, Bwanje valley, lake shore and Phalombe plains as well as dry plateau areas.

Dry cowpea seeds are an important source of affordable protein, B vitamins, and minerals in the predominantly carbohydrate based diet of people in rural communities of southern Africa, Malawi inclusive. Therefore wider utilisation of cowpeas in the diet, presents a source of protein that is within the means of most rural households of Malawi, where protein-energy malnutrition remains a serious public health concern with a 49 % prevalence of stunting among children under-five years of age (National Statistics Office (Malawi), 2004). Inadequate intake of protein in the diet is one of the factors that contribute to such high prevalence of malnutrition in developing countries.

The wider utilisation of dry whole cowpea seeds however is limited due to among other factors, long cooking times and limited variety in cowpea-based products. Boiled dry cowpea seeds (stew) are the main form of consumption in Malawi and other parts of southern Africa such as Botswana (Demooy & Demooy, 1990). The cooking time of cowpeas, which ranges from 35 min to 120 min or more depending on variety and type of cooking water that is used (Olapade, Okafor, Ozumba & Olatunji, 2002), is a great challenge for both urban and rural consumers due to time and energy requirements. Changing life styles in urban areas has placed convenience as a crucial factor in food choices. In addition, fuel wood is a major source of household energy in both urban and rural areas in Malawi and is fast becoming a scarce resource. It has been reported that people adjust their diets in order to optimise the utilisation of energy resources, in that preparation of food items with high energy and time requirement is replaced with fast cooking foods regardless of nutritional value (Brouwer, Hartog, Kamwendo & Heldens, 1996). In this process rural households

may be unable to benefit from available protein resources, such as dry beans and cowpeas, contributing to the proliferation of protein-energy malnutrition.

Micronisation refers to infrared heating and is used as a precooking treatment for the processing of food and animal feed. The name micronisation is derived from the word "micron" the measure used to describe the infrared wavelength (Pickard, 1999). The process uses infrared energy with wavelengths between 1.0 and 3.4 microns to heat water in biological materials by inducing molecular vibrations at a frequency of 8.8 x 10^7 to 1.7 x 10^8 MHz (Cenkowski & Sosulski, 1998). The increased vibrations cause rapid internal heating and rise in water vapour pressure inside the material (Fasina, Tyler, Pickard & Zheng, 1999). Products such as cereal grains can reach internal temperatures of 90 °C in ~50 seconds (Pickard, 1999; Zarkadas & Wiseman, 2001).

The use of hydrothermal treatments such as micronisation of moisture-conditioned seeds has the potential of alleviating long cooking times of grain legumes possibly through the precooking of starch, denaturation of protein, increasing pectin solubility and improving hydration rate of the seeds (Cenkowski & Sosulski, 1997; Arntfield, Scanlon, Malcolmson, Watts, Cenkowski, Ryland & Savoie, 2001; Bellido, Arntfield, Cenkowski & Scanlon 2006). Short cooking time, softer texture and minimal splitting are desirable attributes for cooked dried legume seeds. However, contradictory results have been reported (Abdul-Kadir, Bargman & Rupnow, 1990), where micronisation (17 % moisture, 99 and 107 °C) of pinto beans improved seed rehydration during soaking yet resulted in a 25 % increase in cooking time. The increase in cooking time could be attributed to limited water available during the micronisation process for starch gelatinisation, protein denaturation and pectin solubilisation (Arntfield *et al*., 2001). In the same line, Phadi (2004) postulated that micronisation of cowpeas with 40 % moisture to very high temperatures (160-180 °C) could result in hardening of treated cowpea seeds. It was suggested that high micronisation temperatures (>160 °C) would result in reduced starch gelatinisation during subsequent cooking due to starch degradation and denaturation of the protein matrix that surround the starch granules which would limit hydration of starch granules (Phadi, 2004).

In addition to reducing the cooking time of whole legume seeds, micronisation might affect the functionality of the flour milled from moisture-conditioned and micronised cowpea seeds. Cowpea flour is a versatile food ingredient used in the making of *akara* / *badgia* and *moin moin* (Plahar, Hung, McWatters, Phillips & Chinnan, 2006), bakery (Kerr, Ward, McWatters & Resurreccion, 2001; McWatters, Ouedraogo, Resurreccion, Hung & Phillips, 2003; Hallén, İbanoğlu & Ainsworth, 2004; McWatters, Phillips, Walker, McCullough, Mensa-Wilmot, Saalia, Hung, & Patterson, 2005) and meat products (Prinyawiwatkul, Beuchat, McWatters & Phillips 1997b; Serdaroglu, Yildiz-Turp & Abrodimov, 2005). The suitability of cowpea flour for use in such products is attributed to its functionality in terms of hydration, foaming, gelation, and pasting properties (Prinyawiwatkul, Beuchat, McWatters & Phillips, 1997a; Prinyawiwatkul, McWatters, Beuchat & Phillips 1997c). Protein and starch are the major macromolecules that contribute towards the functionality of cowpea flour. Micronisation has been shown to have an effect on seed hydration, starch and protein (Arntfield *et al*., 2001), which could affect the cooking quality of the cowpeas and the functionality of its flour.

Therefore, it is necessary to determine the structural changes occurring during micronisation of moisture-conditioned cowpea seeds both at molecular and micro structural levels which could lead to changes in physicochemical properties of the seed, its constituent seed coat, protein, starch and functionality of the resultant flour. This information will help in optimising the micronisation processing conditions for cowpeas, in order to produce micronised (moisture-condition, infrared heated) cowpeas of good cooking quality and cowpea flour that could be utilised in other food systems. Thus, through micronisation processing, there is a possibility that cowpeas could be processed into a quick cooking form that would enhance its domestic utilisation hence increase household consumption levels and improve nutritional status.

2 LITERATURE REVIEW

2.1 Utilisation of cowpeas as a protein and energy source in sub-Saharan Africa

Cowpea (*Vigna unguiculata* L. Walp) is a leguminous crop widely grown as an intercrop with cereals in the marginal lands of sub-Saharan Africa. Through improved breeding lines and agronomic practices, there is potential for increased production of cowpeas despite the prevailing production challenges of declining soil fertility, unreliable rainfall, pests and diseases (Singh, Ajeigbe, Ezeaku & Mohammad, 2005). Cowpeas thus will continue to provide an opportunity for an affordable protein source in the carbohydrate-based diet of most people in sub-Saharan Africa.

In Malawi, cowpeas are produced country wide as an intercrop with maize, particularly in warm areas with low rainfall. Locally, cowpeas are known as *Khobwe, Nkhunde, Nseula* and are consumed mainly as boiled dry cowpea seeds, although other products such as fresh or dried leaves (*Ntambe/Chitambe*), green immature pods (*Zitheba*), physiologically mature cowpeas boiled in the pod *(Makata*), decorticated cotyledons (*Chipere:*soaked decorticated, boiled and mashed; usually low quality seeds are used) are also consumed. With the exception of *Makata*, which is consumed as a snack, cowpeas are consumed as relish with the main staple food known as *Nsima* (a thick paste prepared from maize flour).

In West Africa, cowpeas are one of the major sources of proteins in a carbohydrate-based diet (Uwaegbute, Iroegbu & Eke, 2000), where they are consumed in different forms. Whole cowpeas are consumed after being stewed, while different products based on a wet milled paste such as *akara* and *moin moin* are also produced. Apart from the traditional products, cowpeas are also being processed into flour for the production of bakery products such as cookies and breads (Kethireddpalli, Hung, Phillips & McWatters, 2002; McWatters *et al*., 2003; Hallén *et al*., 2004; McWatters *et al*., 2005), as well as comminuted meat products such as chicken nuggets (Prinyawiwatkul *et al*., 1997b) and meat balls (Serdaroglu *et al*., 2005).

2.2 Structure and chemical composition of cowpea seeds

Cowpea are relatively small dicotyledonous seeds (Figure 2.1), ranging in size from 2 to 28.4 g per 100 seeds (Davis, Oelke, Oplinger, Doll, Hanson & Putnam, 1991; Olapade *et al*., 2002; Langyintuo, Ntoukam, Murdock, Lowenberg-Deboer & Miller, 2004). The dimensions of the cowpea seeds are reported to range from 2-12 mm in length (Taiwo, 1998), 6.6 mm in width and 4.4 to 4.9 mm in thickness (Olapade *et al*., 2002; Akinjayeju & Bisiriyu, 2004). Larger seeded cowpeas with rough/wrinkled seed coats are the most preferred by consumers in West Africa (Ehlers & Hall, 1997; Langyintuo *et al*., 2004). Cowpeas in West Africa are manually decorticated and wet milled in order to prepare the different traditional products such as *akara*. As such large seeded cowpeas with rough seed coats are preferred for this purpose because the seed coat is easily removed following brief soaking in water.

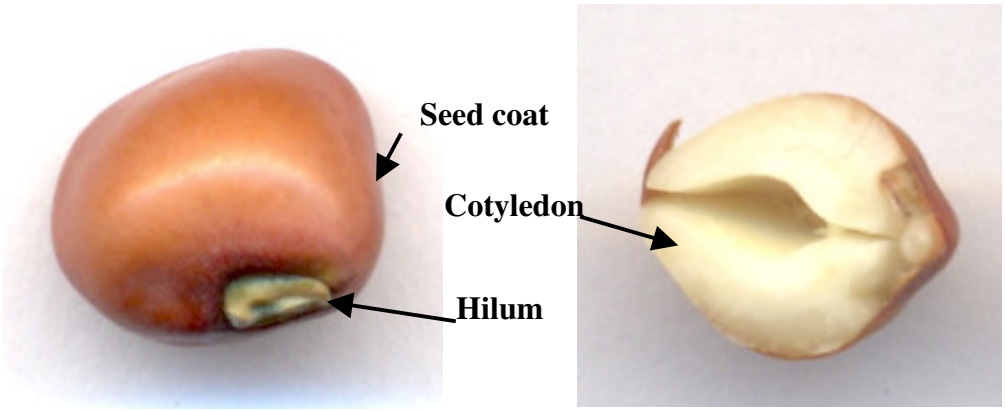

Figure 2.1 Morphology of the cowpea seed showing seed coat, hilum and cotyledon

Cowpea seeds are either kidney or globular shaped (Henshaw, McWatters, Oguntunde & Phillips, 1996; Taiwo, 1998). It has been proposed that the shape of cowpea seeds is dependent on the development process in the pod; kidney shaped seeds develop when there is no space restriction during development within the pod. However, if there is limited space within the pod for the development of the seed, seeds that are globular in shape develop (Davis *et al*., 1991). Cowpea seeds consist of a seed coat,

micropyle, hilum, and the cotyledon (Figure 2.1). Cowpeas are also referred to be eyed (black eye peas) depending on the colouration around the hilum.

2.2.1 Seed coat and other external features of a cowpea seed

The colour of cowpea seeds, which is mainly due to phenolic compounds, varies from white to black and a mixture of the colours in between, some being mottled or speckled (Akinyele, Onigbinde, Hussain & Omololu, 1986; Taiwo, 1998). The seed coats may be loosely or tightly attached to the cotyledon. The texture of the seed coat may be rough, smooth or wrinkled (Olapade *et al*., 2002). East African cowpea consumers prefer seeds with a smooth seed coat, while wrinkled seed coats are preferred in West Africa because it is easy to remove following soaking (Ehlers & Hall, 1997).

The cowpea seed coat comprises of a single or double layer of palisade cells that are elongated along the radial axes of the seed and hour glass cells are found beneath the palisade layer (Sefa-Dedeh & Stanley, 1979b; Lush & Evans, 1980) (Figure 2.2). The thickness, shape and extent of order in the seed coat palisade layer varies among varieties as pointed out by Sefa-Dedeh & Stanley (1979b), in that some varieties have an amorphous cellular layer where the distinct palisade cells can not be identified. The cowpea seed coat contains pectic substances with the following sugar compositions: rhamnose, arabinose, xylose, mannose and glucose (Muralikrishna & Tharanathan, 1994).

Thickness of the cowpea seed coat has been reported to range from 5.84 to 59.33 µm (Sefa-Dedeh & Stanley, 1979b). Thinner seed coats in cowpeas have been shown to enhance rapid softening during soaking (Sefa-Dedeh, Stanley & Voisey, 1978) as compared to relatively thicker seed coats especially those that have a waxy layer (Sefa-Dedeh & Stanley, 1979a).

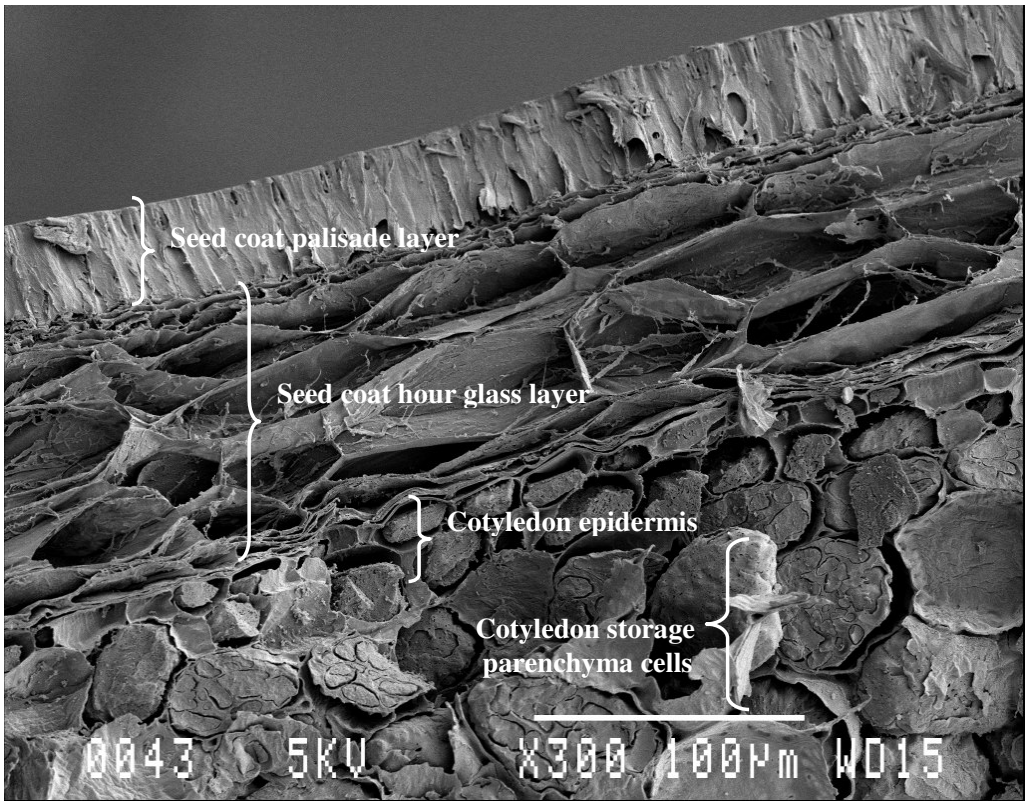

Figure 2.2 **Cross section of a dry Bechuana white cowpea (*Vigna unguiculata*) seed** (Phadi, 2004)

The seed coat is the first physical entity facing the transportation of water into the cotyledon. In addition to the wide variations in seed coat thickness, the seed coat percentage in relation to the whole seed has been reported to range from 1.5 to 16 % (Akinyele *et al*., 1986; Olapade *et al*., 2002). Such variation in seed coat must play an important role in determining the rate of water uptake during soaking (Sefa-Dedeh & Stanley, 1979b).

Apart from the seed coat, other external features of the cowpea seed, i.e. the micropyle, hilum and raphe also contribute in the process of water imbibition (Lush & Evans, 1980). Sefa-Dedeh & Stanley (1979b) reported that cowpeas have an elliptical hilum (2.1 to 3.0 mm) with a micropyle situated below it. The micropyle was reported to be y-shaped and closed in some cowpea varieties and circular and open in other varieties.

2.2.2 Structure and physicochemical characteristics of cowpea cotyledon

The cotyledon is the major storage structure in cowpea seeds with carbohydrates and proteins being the major constituents (Table 2.1). The outer surface of the cowpea cotyledon appears to have wide "hills" and narrow "valleys" while the cotyledon cross section distinctly shows the presence of storage parenchyma cells ranging in length from 80-120 µm and 50 -90 µm in width (Liu, Hung & Phillips, 1993a).

Table 2.1 Chemical composition of whole cowpea seeds (Longe, 1980)

Constituent	Percent (%)	Sugars	Percent (%)
Moisture	10.4	Glucose	0.2
Crude protein	28.0	Fructose	0.4
Fat extract	1.9	Sucrose	1.6
Ash	3.8	Raffinose	0.7
Crude fibre	3.1	Stachyose	2.7
Starch	40.6	Verbascose	3.6

The cowpea cotyledon parenchyma cells are filled with starch granules, protein bodies and a cytoplasmic matrix (Figure 2.3) (Sefa-Dedeh *et al*., 1978; Hung, McWatters, Phillips & Chinnan, 1990; Phadi, 2004) with lipid bodies mainly found along the cell wall (Saio & Monma, 1993). The cytoplasmic matrix in cowpea cotyledon consists of protein and minute levels of lipid material as indicated by a faint staining with Sudan Black (Saio & Monma, 1993).

2.2.2.1 Physicochemical and functional characteristics of cowpea protein

Proteins are macromolecules composed of 20 α-amino acid residues in the L-configuration (Charley & Weaver, 1998). The protein content of cowpeas has been reported to range from 21.70 to 30.32 %, based on variety and agronomic conditions (Akinyele *et al*., 1986; Chan & Phillips, 1994; Aluko & Yada, 1995; Mwasaru, Muhammad, Bakar & Che-Man, 1999a). Protein exists in cowpea grains as protein bodies and as a part of the cytoplasmic matrix that enclose starch granules (Liu *et al*., 1993a).

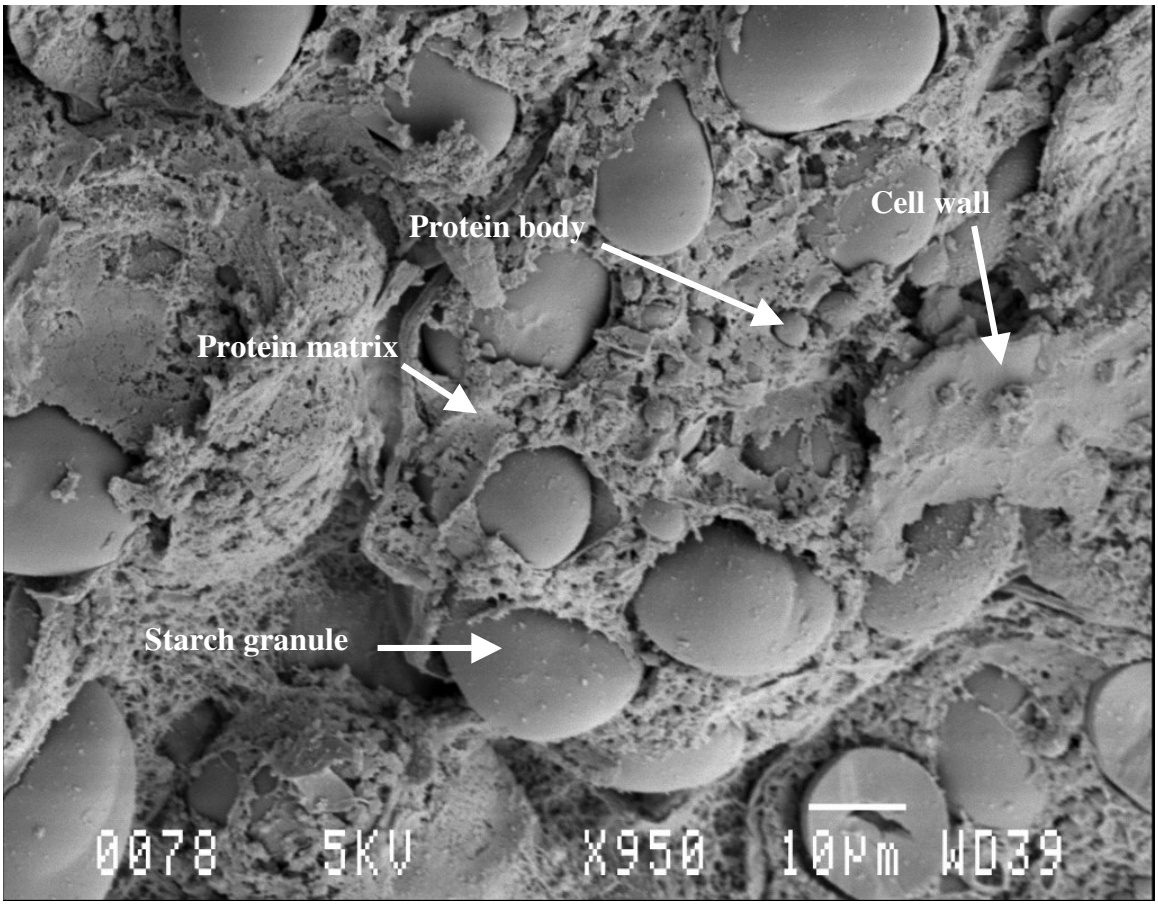

Figure 2.3 **Cross section of tempered (41 % moisture) Bechuana white cowpea seed cotyledon** (Phadi, 2004)

The diameter of cowpea protein bodies is reported to range from 2 to 6 µm (Harris & Boulter, 1976; Saio & Monma, 1993). Plant storage proteins are classified as albumins, globulins, prolamins and glutellins according to their solubility in water, salt solution, alcohol and alkali, respectively (Chan & Phillips, 1994). The size of these fractions in cowpea protein varies widely owing to differences in genetic attributes of samples and methods of extraction and determination (Chan & Phillips, 1994; Oliveira, Pinto, Vasconcelos, Fernandes, Ramos, Ferreira & Rios, 2004) (Table 2.2).

Globulins are the major cowpea seed proteins ranging from 48.2 to 90 % (Chan & Phillips 1994; Freitas, Teixeira & Ferreira, 2004) of the total proteins. The glutellin and prolamin fractions in cowpeas range from 5.16 - 6.74 % and 0.8 – 1.05 %, respectively (Chan & Phillips, 1994; Freitas *et al*., 2004).

Table 2.2 Cowpea protein fraction sub unit composition and molecular properties

Protein fraction	Proportion of total protein (%)	Subunit composition (Molecular weight in kDa)	Disulphide bonds	Glycosylation
CPI*	12[a]	150, 50, 52, 30	None	ND
	ND	Major[b]: 40, 60, 66	ND	ND
		Minor[b]: 30	ND	ND
Albumins	24.9[c]	Major[c]: 99, 91, 32, 30	None	None
		Minor[c]: 28, 83-52	None	None
	45[d]	ND	ND	ND
	10.2[e]	4 bands :27 - 30	None	ND
		4 bands :81 - 93	None	ND
Globulins	66.6[c]	Major[c]: 65, 60, 56, 50	None	60, 56, 50 [c]
		Several minor[c]:28 - 42	None	29[c]
	51[d]	α- Vignin [d]		
		Major:80 (60+20)	Interpolypeptide	None
		Minor: 44-58	None	None
		β- Vignin [d]		
		Major: 55, 60	None	Glycosylated
		Minor: 22,24,30,66	None	None
		γ – Vignin[d] :Major: 22	Intrapolypeptide	None
Glutellins	4.7[c]	101, 68, 31, 29[c]	None	None
	3[d]	ND	ND	ND
Prolamins	0.7[c]	105, 62, 59, 54[c]	None	None
	1[d]	ND	ND	ND

CPI - Cowpea Protein Isolate; * Percent of defatted cowpea flour; ND = Not determined, [a]Rangel, Domont, Pedrosa & Ferreira (2003); [b]Horax, Hettiarachchy, Chen & Jalaluddin (2004a); [c]Chan & Phillips (1994); [d]Freitas *et al*. (2004); [e]Oliveira *et al*. (2004)

While reports on the distribution of glutellins and prolamins in cowpea seeds are consistent, wide variations exist on the distribution of albumin and globulin fractions. Ragab, Babikar & Eltinay (2004) used water to isolate cowpea albumins and reported that the albumin fraction was 71 % of the total cowpea protein. Such high albumin fractions could possibly be due to contamination of the albumin fraction with globulins, leading to underestimation of the latter. Based on the observation that Ca^{2+} and Mg^{2+} would form electrostatic bridges between negatively charged globulin molecules, leading to self aggregation of globulins from solution (Ferreira, Franco & Teixeira, 1999), Freitas *et al*. (2004) used pH 8 water containing Ca^{2+} and Mg^{2+} cations to extract the albumin fraction, hence reducing cross contamination.

The cowpea globulin is a 7S vicillin type glycoprotein also known as vignin and it is reported to be composed of three main units designated α, β, and γ vignin (Aluko & Yada, 1995; Freitas *et al*., 2004) while Chan & Phillips (1994) reported 4 major subunits. Globulins are defined as the proteins that are insoluble in water or low salt solutions but readily soluble in solutions of high ionic strength due to calcium magnesium dependent self aggregation (Ferreira, Freitas & Teixeira, 2002). Under native conditions α–vignin was reported to be highly mobile towards the anode and strongly binding to the anion exchanger. α–vignin is composed of a major 80 kDa subunit and several smaller (44-58 kDa) subunits linked by disulphide bonds (Freitas *et al*., 2004). β–vignin is composed of glycosylated polypeptides, two major ones (55 and 60 kDa) and several minor (22, 24, 30 and 66 kDa). β–vignin does not contain disulphide bonds. γ–vignin is a minor vigna globulin composed of a 20 kDa polypeptide with an intrapolypeptide disulphide bond (Freitas *et al*., 2004).

Chan and Phillips (1994) reported that cowpea albumins had subunits with molecular mass 99, 91, 32 and 30 kDa in addition to less prominent polypeptides with molecular mass around 28 kDa. Similar molecular band distribution for cowpea albumin was reported by Oliveira *et al*. (2004). Chan and Phillips (1994) separated the glutellin fraction into several bands with molecular masses 101, 68, 31, and 29 kDa and others in a molecular mass range of 62-44 kDa. Four dominant bands of molecular masses 105, 62, 59 and 54 kDa were found in the prolamin fraction and it had the highest amount of hydrophobic amino acids among the flour protein fractions (Chan & Phillips, 1994).

Cowpea protein is relatively hydrophilic as shown by the amino acid profile (Table 2.3). Horax *et al*. (2004a) reported that cowpea protein has a lower surface hydrophobicity in comparison to soy protein isolate. The lower surface hydrophobicity of cowpea proteins indicates that most of the amino acids with non-polar/hydrophobic side chains in the polypeptides chains are buried in the interior of the protein. This hydrophilic character of cowpea protein would promote protein water interactions and account for the greater than 80 % solubility in water at pH 6 and above (Horax, Hettiarachchy, Chen & Jalaluddin, 2004b; Horax *et al*., 2004a).

Table 2.3 Amino acid profile of decorticated Bechuana white cowpea flour (Abu, Müller, Duodu & Minnaar, 2005)

Amino acid	Cowpea flour (g/100g)	Amino acid	Cowpea flour (g/100g)
Acidic side chains		**Basic side chains**	
Aspartic acid	2.76	Histidine	0.73
Glutamic acid	4.29	Arginine	1.69
		Lysine	1.66
Non-polar side chain			
Alanine	1.10	**Polar side chains**	
Proline	1.03	Tryptophan	0.08
Valine	1.12	Serine	1.41
Methionine	0.35	Threonine	1.06
Isoleucine	0.93	Tyrosine	0.68
Leucine	1.84	Cysteine	0.23
Phenylalanine	1.38		
Glycine	0.93		

The high water solubility of cowpea protein has been associated with good foaming and water absorption properties of cowpea flour. Cowpea protein is relatively heat stable compared to soy protein with denaturation temperature ranging from 78 ºC to 88 ºC (Horax *et al*., 2004a; Abu, Müller, Duodu & Minnaar, 2006a).

2.2.2.2 Physicochemical and functional characteristics of cowpea starch

Cowpeas contain approximately 48 % starch (Kerr *et al*., 2001). Starch is a macromolecule consisting of D-glucopyranose linked together by α-1, 4 and α-1, 6 glycosidic bonds (Thomas & Artwell, 1999). The polymerisation of D-glucopyranose results in two types of starch polymers, namely amylose and amylopectin. Amylose is a linear polymer held together by α-1, 4 glycosidic bonds while amylopectin is a branched polymer of short chains (DP 6- 50 glucopyranose residues) linked by α-1, 6 glycosidic bonds to long chains (DP 50-80 glucopyranose residues) of glucose moieties linked through α-1, 4 bonds (Biliaderis, 1991; Thomas & Artwell, 1999; Huang, Schols, van Soest, Jin, Sulman & Voragen, In press). The cowpea amylopectin profile has a weight ratio for short: long chain of 3.1:1 (Huang *et al*., In press). The molecular weight of the starch polymers ranges from 1.5 x 10^5 to 10^6 for amylose, and 50-500 x 10^6 kDa for amylopectin, respectively (Billiaderis, 1991). Hence amylopectin is considered to be one of the largest naturally occurring biopolymers (BeMiller & Whistler, 1996). The amylose and amylopectin content of starches significantly influence its functional properties. The amylose content of cowpea varieties has been reported to range from 6.92 to 39.30 % with an average of 17.73 % (Akinyele *et al*., 1986; Aremu, 1991). Such variation in amylose/ amylopectin ratios would most likely have an effect on cooking characteristics of cowpeas such as water absorption during cooking.

Microstructural examination of cowpea cotyledon cells using scanning electron microscopy (SEM) indicates that cowpea starch exist as singular granules enveloped by a protein matrix (Liu *et al*., 1993a; Phadi, 2004). The shape of cowpea starch granules is reported to be morphologically irregular, with some granules being oval, ellipsoidal or kidney shaped (Saio & Monma, 1993). The granules appear to have cracks on the surface. These cracks have been observed in both sun dried and hot air dried cowpea starch. The length of cowpea starch granules range from 7.5 to 37.5 µm and the widths range from 5 to 27.5 µm (Saio & Monma, 1993; Agunbiade & Longe, 1999).

Starch granules have a semi crystalline structure which gives distinct X-ray diffraction patterns used in classifying starch into A-type found in cereals, B- type found in root and tubers and the C type, which is an intermediate between A and B types and that is

found in legume starch such as cowpea starch (Biliaderis, 1991; Huang *et al*., in press). The crystallinity of native starch granules is derived from the packing of amylopectin short chains.

Gelatinisation refers to the disruption of granular structure, hydration, swelling and solubilisation of starch molecules, in the presence of adequate water and heat (Biliaderis, 1991). Gelatinisation of starch is a complex process, and a number of methods have been employed to understand the various changes that occur in the starch granule. Granular swelling is one of the initial changes that occur during the gelatinisation of starch and the onset/pasting temperature is generally used to indicate this stage. The onset/pasting temperature is characteristic of each starch source and process history. The gelatinisation temperature of cowpea starch has been reported to range from 67-78 °C (Agunbiade & Longe, 1999; Abu, Müller, Duodu & Minnaar, 2006b). Gelatinisation temperature measured using DSC is an indication of the endothermic transition involved in the melting of the crystalline structure of the starch granules and is also evidenced by loss in birefringence. Cowpea starch is reported to exhibit either B (moderate swelling) type of swelling at higher concentration (7-8 %) and/or C (restricted swelling) type at lower concentration (4-6 %) (Prinyawiwatkul *et al*., 1997c). Cowpea starch granules heated under excess moisture were shown to increase in diameter by 258 % (Rao, Okechukwu, Da Silva & Oliveira, 1997). The peak swelling volume of cowpea starch has been reported to range from 18 to 20.7 ml g^{-1} (Prinyawiwatkul *et al*., 1997c; Abu *et al*., 2006b). Regardless of starch concentration, swollen cowpea starch granules are reported to be resistant to mechanical disintegration during cooking and agitation as indicated by low shear thinning values (Prinyawiwatkul *et al*., 1997c; Huang *et al*., in press). Upon cooling, cowpea starch paste has a high tendency of retrogradation resulting in the formation of a gel (Henshaw *et al*., 1996; Henshaw, McWatters, Akingbala & Hung; 2002) possibly due to the high amylose content of cowpea starch.

2.3 Mechanisms underlying structural and physicochemical changes of legume seeds during soaking

Cooking of cowpeas is a form of hydrothermal processing that involves hydration and heating which may take place separately or concurrently (Sefa-Dedeh *et al*., 1978). Hydration properties of the seeds at room temperature such as hydration rate and hydration capacity are generally used to indicate cooking properties. Hydration rate of leguminous seeds has mainly been attributed to structural characteristics of the seed coat and cotyledon, while protein content and other macromolecules such as cell wall material and pectins affect hydration capacity (Sefa-Dedeh & Stanley, 1979b). Hydration capacity is a reflection of the water holding capacity of the seeds which includes water of hydration as well as capillary water. Sefa-Dedeh & Stanley (1979b) proposed that water uptake in cowpeas could be a sequential process involving seed coat structure and thickness during the initial soaking stage (< 3 h), seed size /volume and hilum size in the intermediate stage (3-6 h) and protein content (>12 h) in the final stages of soaking.

The cowpea seed coat is the part in direct contact with water during soaking and is one of the conduits for water to the cotyledon. Variations in cowpea seed coat structural characteristics and mode of attachment to the cotyledon have been documented (Sefa-Dedeh & Stanley, 1979a; Sefa-Dedeh & Stanley, 1979b; Lush & Evans, 1980). Amorphous and thin seed coats have been shown to promote higher rate of seed hydration during soaking as compared to the more organised palisade cells found in thicker seed coats (Sefa-Dedeh *et al*., 1978; Sefa-Dedeh & Stanley, 1979b; Lush & Evans, 1980). At the same time, some cowpea seed coats have a waxy layer on the top side which was reported to contribute towards delayed hydration in those cowpea varieties (Sefa-Dedeh & Stanley, 1979a). Apart from the seed coat structural differences, Olapade *et al*. (2002) reported that cowpeas with a tightly attached seed coat absorbed less water than cowpeas with moderately attached seed coats.

In addition to the seed coat characteristics, the seed cotyledon structure has been reported to affect hydration rate. Sefa-Dedeh & Stanley (1979b) reported that cowpea varieties with porous cotyledons had higher rates of hydration than seeds with compact cotyledons. Water imbibition by the cotyledon involves the physical

movement of water to fill in the inter-cellular spaces (Phlak, Caldwell & Stanley, 1989). Hence loosely packed cotyledon parenchyma cells would provide easier access for water as compared to compactly packed cells.

With increasing soaking time the cotyledon properties have been reported to play a major role in the hydration process. Cowpea seed protein content was reported to be the main factor affecting water absorption during extended soaking (>12 h) (Sefa-Dedeh & Stanley, 1979b). Cowpea protein has been reported to be relatively hydrophilic (Horax *et al*., 2004a) and thus plays a major role in hydration of the seed (Sefa-Dedeh & Stanley, 1979b). Water is held by proteins through the formation of hydrogen bonds with the hydrophilic polar side chains. The hydration capacity of cowpea seeds has been reported to range from 1.14 to 1.60 g g^{-1} (Olapade *et al*., 2002) and it is positively related to protein content.

Sefa-Dedeh *et al*. (1978) reported a positive correlation between water absorption during soaking and cooked cowpea texture, suggesting that it could be used to predict cooked seed texture. Although there is wide variation in hydration rate and capacity of cowpeas during soaking, a concrete relationship with cooking time and texture has not been firmly established.

2.4 Mechanisms underlying structural and physicochemical changes of legume seeds during cooking

Whole dry cowpeas are usually boiled in water in order to tenderise the cotyledons and develop a cooked flavour resulting in increased palatability of the product (Aremu, 1991). The amount of time required for cowpeas to attain a soft cooked texture and acceptable flavour varies (30 to 160 min) depending on factors such as, variety, pre-treatment of the seeds such as soaking, and cation content of the cooking water (Akinyele *et al*., 1986; Taiwo, 1998). Cooking time is thus a very important quality parameter for whole cowpeas. In general, cooking time is measured relative to the softening of the cotyledon texture rather than flavour attributes, which would include cooked aroma (Proctor & Watts, 1987a; Phadi, 2004). Thus the process of cooking dry legume seeds to a soft texture is mainly characterised by cotyledon

parenchyma cell separation, protein denaturation and starch gelatinisation (Sefa-Dedeh *et al*., 1978; Sefa-Dedeh & Stanley, 1979a).

2.4.1 Physicochemical and structural changes occurring in the middle lamella of parenchyma cells of the cowpea cotyledon

Solubilisation of the middle lamella has been shown to result in cell separation during cooking, consequently leading to a soft cooked texture (Sefa-Dedeh *et al*., 1978; Sefa-Dedeh & Stanley, 1979a). The mechanism underlying cell separation involves the heat-catalysed depolymerisation of the middle lamella pectin polymers. The depolymerisation involves beta elimination of the methyl esterified polygalacturonic acid (Liu, Phillips & McWatters, 1993b; Brett & Waldron, 1996). When cowpeas are cooked at 100 °C, the middle lamella is weakened resulting in cell separation. Several researchers have reported cell separation along the middle lamella after boiling of cowpea seeds (Sefa-Dedeh *et al*., 1978; Sefa-Dedeh, Stanley & Voisey, 1979; Liu *et al*., 1993b). A similar phenomenon has also been reported in cowpeas that have been exposed to a pre-decortication drying treatment, which involved drying of cowpea seeds at 130 °C from 25 % moisture content to 11 % (Hung *et al*., 1990).

2.4.2 Gelatinisation of starch and protein denaturation during cooking of cowpea seeds

Gelatinisation of starch during cooking of cowpea seeds is one of the major physicochemical and structural changes occurring in the cotyledon cell that contributes to the softening of cowpea seeds (Sefa-Dedeh *et al*., 1979; Liu *et al*., 1993a). During gelatinisation, the starch granules absorb water and swell up, losing their crystalline and glassy state in the process. Due to granular swelling, amylose may leach out of the granules and associate outside the granule. This chain of events contributes towards the softer texture of cooked legume seeds. Although significant variations have been reported in starch and amylose content of cowpea varieties, no definite correlation with cooking time of cowpeas has been found (Akinyele *et al*., 1986; Aremu 1991). However, Aremu (1991) reported a significant ($r = 0.73$) correlation between amylose content and the time required for the cowpeas to attain maximum swelling (water uptake during cooking). The maximum swelling of cowpeas during cooking was approximately 30 min after the acceptable cooking time.

These results indicate that the gelatinised starch granules at the stage when the cowpeas were considered cooked were still capable of absorbing more water.

Akinyele *et al*. (1986) reported an increasing trend in the cooking time of cowpeas with increasing protein content. Cowpea protein is relatively hydrophilic (Mwasaru *et al*., 1999a) and it has been shown to absorb approximately 1.24 g of water per g of protein (Mwasaru, Muhammad, Bakar & Che Man, 1999b). The hydrated native proteins in cowpeas facilitate the hydration process of the cowpea cotyledon cells. The denaturation temperature of cowpea protein ranges from 78 to 88 °C (Horax *et al*., 2004a; Abu *et al*., 2006a). Protein denaturation involves the unfolding of the protein molecule and possible increase in exposed hydrophobic sites leading to the formation of aggregates (Clark & Lee-Tuffnell, 1986) and possibly formation of a thermally induced gel. Horax *et al*. (2004b) reported that cowpea protein could form a gel after 10 min of heating at 90 °C. This would imply that cowpea seeds could be heated in water at 90 min for almost 10 min, before protein gelation could take place. This is an important observation since protein gelation and starch gelatinisation require water, yet the starch is imbedded in the protein matrix (Figure 2.3). Since the gelatinisation temperature of cowpea starch is lower than the denaturation temperature of cowpea protein, there is minimal competition for water between starch gelatinisation and protein gelation. It has been observed that in hard to cook cowpeas the denaturation temperature of cowpea protein is reduced to approximately 58 °C due to increased tissue acidity (Liu, McWatters & Phillips, 1992). The decrease in tissue acidity of aged seeds has been attributed to hydrolysis products of fatty acids, phytates and storage proteins (Hohlberg & Stanley, 1987). The denaturation temperature observed in protein from HTC seeds was lower than the gelatinisation temperature of cowpea starch (Liu *et al*., 1992). Due to this change in protein thermal properties, it has been suggested that the gelation of protein would out compete starch gelatinisation for water, resulting in limited starch hydration and gelatinisation and consequentially into a hard texture.

A soft cooked texture is an important quality characteristic in cooked cowpeas. The overall texture of cooked cowpeas is a composite of the characteristics of the seed coat and the cotyledon. Decorticated beans require shorter time to attain cooked texture. Jackson & Varriano-Marston (1981) demonstrated that the seed coat made a 38 %

contribution towards the cooking time (Mattson bean cooker) of beans. Demooy & Demooy (1990) observed that some cowpeas had a tough seed coat even though the cotyledon was thoroughly cooked, while in some varieties the seed coat and cotyledon were all mushy. This difference in seed coat texture could be attributed to varietal differences in structure (thickness and order) and composition (Sefa-Dedeh & Stanley, 1979b; Lush & Evans, 1980). Smaller seeded cowpeas that were used in the study by Demooy & Demooy (1990) disintegrated into small soft pieces (grainy mouthfeel) as compared to the smooth mouthfeel observed for the mushy varieties possibly due to differences in amylose/amylopectin ratios. Cowpea varieties that have high amylopectin content (Akinyele *et al*., 1986) would possibly have a mushy texture as compared to that of varieties with high amylose content, since the high amylose would retrograde during cooling to form a firm gel which would not be the case in high amylopectin seeds. Another contributing factor to a grainy mouthfeel in cooked cowpea could be due to differences in protein content. Akinyele *et al*. (1986) observed that the cooking time of 18 cowpea varieties increased with increase in protein content. This may imply that high protein content could increase the competition for water between starch gelatinisation and gelation and in the process limit hydration of starch. The poor hydration of the starch would result in a grainy texture.

2.4.3 Splitting of cowpea seeds during cooking

Cooked cowpeas tend to split and form lumps. Splitting and lumping of cooked cowpeas are usually regarded as undesirable characteristics (Taiwo, Akanbi & Ajibola, 1997a; Afoakwa, Yenyi & Sakyi-Dawson, 2006). In some cases these two phenomena result in empty seed coat shells with its contents in the cooking water. Cowpeas split in two main different ways. Splitting either starts transversely on the seed coat below the hilum, followed by the cotyledon, while in other cases the split is longitudinal, resulting in separation of the two cotyledons (Taiwo, Akanbi & Ajibola, 1997b). Although no concrete hypothesis has been put forward to explain splitting and lumping in cooked cowpeas, factors such as variety, pre-treatment and final water uptake have been associated with splitting. Some cowpea varieties are more prone to splitting than others, possibly due to differences in physicochemical properties. Starch and protein are the main water absorbing and holding entities during cooking of cowpea seeds. Taiwo, Akanbi & Ajibola (1998) reported that splitting of cooked

cowpeas correlated positively with drained weight and softness (penetration depth). Seed softness and drained weight have also been related to starch gelatinisation. Gelatinisation characteristics of starch are dependent on its amylose/amylopectin ratios. Akinyele *et al*. (1986) reported a negative correlation between swelling capacity (hydration during cooking) of cowpeas and amylose content. Therefore, it is possible that varietal differences in starch and protein could contribute towards splitting in cowpea during cooking. However the current literature on cooking characteristics of cowpea seeds does not elaborate on the underlying physicochemical and structural factors for the splitting phenomenon.

2.5 Functional properties of cowpea flour

Functional properties refer to physical and chemical properties of a food or food component which affect utilisation with the exception of nutritional attributes (Zayas, 1997). Functional properties of a food ingredient determine its suitability for use in food systems. Cowpea flour has been used both as a major and minor ingredient in various food systems, such as *akara, moin moin* (Phillips, McWatters, Chinnan, Hung, Beuchat, Sefa-Dedeh, Sakyi-Dawson, Ngoddy, Nnanyelugo, Enwere, Komey, Liu, Mensa-Wilmot, Nnanna, Okeke, Prinyawiwatkul & Saalia, 2003) and comminuted meat products (Prinyawiwatkul *et al*., 1997b; Serdaroglu *et al*., 2005). Utilisation of cowpea flour in food systems presents an opportunity for extending the use of cowpeas beyond the whole seed. The functionality of cowpea flour in such food systems depends on its physical properties such as milling (Kerr *et al*., 2001; Singh, Hung, Corredig, Phillips, Chinnan & McWatters, 2005) and its major macromolecular constituents namely protein and starch.

Cowpea protein has been associated with water solubility, gelation, fat absorption, water holding capacity and viscosity while starch is associated with swelling, viscosity and gelatinisation. However in heterogeneous systems such as cowpea flour, these functional properties are affected by an interaction of the major macromolecules as well as minor constituents.

2.5.1 Nitrogen solubility of cowpea flour

Most of the protein related functional properties are dependent on its solubility in water. The solubility of a protein in an aqueous solvent represents a thermodynamic equilibrium existing between protein-protein and protein-solvent interactions (Damodaran, 1996a). This is influenced by amino acid composition, sequence, molecular weight and conformation (Zayas, 1997). Protein solubility is generally expressed as nitrogen solubility index (NSI), which refers to the total nitrogen in aqueous solution or dispersion, which does not sediment due to moderate centrifugal force (Zayas, 1997). Native cowpea protein is considerably soluble in water due to its largely hydrophilic amino acid profile (Table 2.3) (Mwasaru *et al*., 1999a). The NSI of cowpea flour is reported to be higher at alkaline pH than acidic conditions (Prinyawiwatkul *et al*., 1997a). Solubility of most proteins is reduced when severe heat treatment is applied. Thermal treatment (soaking and boiling for 45 min) of cowpea seeds has been reported to cause 48 – 80 % reduction in protein solubility possibly due to the unfolding of the protein units resulting in the exposure of hydrophobic groups (Prinyawiwatkul *et al*., 1997a). The exposed hydrophobic groups interact with each other leading to aggregation of the unfolded molecules (Giami, 1993; Prinyawiwatkul *et al*., 1997a).

2.5.2 Foaming capacity of cowpea flour

Foaming in protein stabilised aqueous systems reflects the proteins' ability to form stable layers surrounding gas droplets in a liquid phase (Rangel *et al*., 2003). This requires the protein to be soluble in the aqueous phase and be in a position to diffuse and concentrate at the air/water interface and partially unfold to form a cohesive layer around the gas bubbles as well as possess sufficient viscosity and mechanical strength to prevent rupture and coalescence of droplets (Damodaran, 1996a). In cowpea flour, foaming capacity of cowpea paste is desirable for the development of textural properties and mouthfeel of foam type products such as *akara / badgia* (Plahar *et al*., 2006). The foaming capacity of cowpea flour has been reported to range from 44 – 80 % (Olaofe, Umar & Adedirani, 1993) and was positively correlated to protein solubility. Due to their surfactant properties, saponins have been shown to improve the foaming capacity (measured as specific gravity) of cowpea pastes made from cowpea flour (Park, Plahar, Hung, McWatters & Eun, 2005). Since foaming involves the incorporation of air into a paste, it results in lowering of specific gravity.

2.5.3 Water holding capacity of cowpea flour

Water holding capacity has been defined as the ability of a flour to hold its own and added water during the application of force, pressing, centrifugation or heating (Zayas, 1997). Water holding capacity is one of the hydration properties of flour that would determine its use in food systems such as comminuted meat products, baked dough and steamed pastes. The water absorption capacity of whole cowpea flour has been reported to range from 2.6 to 2.98 g g^{-1} flour (Giami, 1993; Olaofe *et al*., 1993). The main water absorbing component in cowpea flour is protein through the formation of hydrogen bonds with the hydrophilic polar side chains. In addition other components such as starch and cell wall material also contribute to water holding capacity of cowpea flour. Agunbiade and Longe (1999) reported that cowpea starch has a 94 % water absorption capacity which would contribute towards hydration of the flour. Cell wall material (CWM) is generally composed of cellulose, hemicellulose and pectins, which form a matrix structure where capillary water is held, contributing towards water holding capacity of the flour. Kethireddipalli *et al*. (2002) reported that CWM isolated from finely milled cowpea flour absorbed 10.04 g of water g^{-1} of freeze dried CWM.

2.5.4 Fat binding capacity of cowpea flour

The ability of proteins to bind fat is important in food systems, since fats and oils act as flavour retainers and contribute to mouthfeel in comminuted meat (Prinyawiwatkul *et al*., 1997b; Serdaroglu *et al*., 2005) and baked products (Phillips *et al*., 2003). Oil absorption in proteins and other food components is attributed mainly to physical entrapment (Zayas, 1997). Proteins through the non polar side chains form hydrophobic interactions, electrostatic, hydrogen and covalent bonds with oil, consequently facilitating oil absorption (Zayas, 1997). As a result, oil absorption capacity has been reported to correlate positively with protein surface hydrophobicity and negatively with protein solubility. Giami (1993) reported that cowpea flour (2.8 g g^{-1} flour) had higher fat binding capacity than raw soy flour and winged bean flour. The fat absorption capacity of the cowpea flour increased following thermal treatment (3.1 g g^{-1}) (Giami, 1993). However, Prinyawiwatkul *et al*. (1997a) reported a lower (0.69 %) oil retention capacity for cowpea flour, which would be most likely, given the hydrophilic characteristic of cowpea protein. Thermal treatment slightly increased the OAC of the flour possibly due to increased surface hydrophobicity of the proteins,

which has been associated with the unfolding of proteins when exposed to heat (Prinyawiwatkul *et al*., 1997a).

2.5.5 Pasting and gelling properties of cowpea flour

Pasting properties are important characteristics for starchy flours. Pasting curves derived by a rapid visco-analyser (RVA) or Brabender viscoamylograph have been used to study the gelatinisation behaviour of raw and processed cowpea flour (Henshaw *et al*., 1996; Prinyawiwatikul *et al*., 1997c). Pasting curves display the different stages of the gelatinisation process from granular swelling through to gelation upon cooling. The characteristics of the hot and cold paste of the cowpea flour would affect the textural properties of end products. Wide variations have been reported in the viscosities of cowpea flour due to variations in variety and whether the flour was milled from decorticated or whole seeds (Henshaw *et al*., 2002).

Cowpea flour pastes have been reported not to exhibit a peak, and the pastes formed were stable during heating and had high set back values (Henshaw *et al*., 1996; Henshaw *et al*., 2002). Set back values are an indication of the ability of the dispersed amylose to re-associate and form a gel. However the formation of a gel in heterogeneous systems is a factor of thermally-induced protein gelation and gelling of gelatinised starch. Cowpea flour has a least gelling concentration that range from 10 to 15 % (Olaofe *et al*., 1993; Prinyawiwatkul *et al*., 1997a). The variations in gelling properties among cowpea flours could be due to differences in concentration and composition of gelling macromolecules i.e. protein and amylose in the flour. The protein and starch content of cowpea flour would vary among different cowpea varieties as well as due to processing, i.e. whether the flour was milled from whole or decorticated seeds.

2.5.6 Thermal properties of cowpea flour

Thermal treatment is one of the common process steps in food processing and utilisation. As such, thermal properties of a food or its ingredients are important determining factors of its functionality, since structural parameters that influence functionality may be altered chemically during the application of heat. Thermally induced changes in the state of substances are accompanied by change in energy level that is manifested through either absorption or dissipation of energy (Kolbe, Wilson &

Hartel, 1999; Yu & Christie, 2001). Thermal transitions in cowpea flour have been attributed to protein denaturation and starch gelatinisation (Henshaw, McWatters, Akingbala & Chinnan, 2003) and this would appear as two endothermic peaks. However, Henshaw *et al*. (2003) reported that most cowpea varieties that were analysed exhibited a single endotherm (~ 80.9 °C), which was attributed to starch gelatinisation while the apparent endotherm for protein denaturation at a higher temperature (90 °C) was evident in few varieties. Since the endothermic curve is a combination of multiple endothermic and exothermic peaks, what appears as a single peak could in fact be several peaks especially since there is an overlap in the temperature range reported for cowpea starch gelatinisation (67 to 78 °C) (Agunbiade & Longe, 1999; Abu *et al*., 2006b) and protein denaturation (78 to 88 °C) (Horax *et al*., 2004a; Abu *et al*., 2006a).

2.6 Use of micronisation to precook grain legumes

Micronisation refers to a short-time and high-temperature infrared processing method that utilises moisture, temperature and mechanical pressure to achieve conditions for optimum cooking. During micronisation of leguminous seeds, heat is generated inside the seed, rapidly vaporising the water and increasing pressure inside the seed that may lead to rupturing of the seed coat (Fasina, Tyler, Pickard, Zheng & Wang, 2001). The combination of high temperature and pressure during micronisation is crucial in the structural changes that occur in micronised seeds (Wang, McAllister, Zobel, Pickard, Rode, Mir & Cheng, 1998).

Infrared heating is a dry heat process and it requires the presence of moisture in the material being processed. Thus micronisation processing may include conditioning of grains to increase moisture content and holding to allow the moisture to equilibrate throughout the seed. Moisture is necessary for generation of heat as well as heat transfer and chemical and structural changes that accompany heat treatment of moisture conditioned seeds, namely starch gelatinisation, protein denaturation and solubilisation of the middle lamella between parenchyma cotyledon cells (Liu *et al*., 1993a; Liu *et al*., 1993b; Arntfield, Scanlon, Malcolmson, Watts, Ryland & Savoie, 1997).

2.6.1 Effect of micronisation on cooking characteristics of dry legume seeds

Cooking time is one of the major criteria used to evaluate the food quality of dry legume seeds such as cowpeas (Proctor & Watts, 1987a). Most of the methods used to measure cooking time are based on texture (hardness); hence cooking time is discussed as the time required by the seeds to attain a softer texture. Micronisation of moisture-conditioned seeds has generally been reported to reduce the cooking time of legumes by 50 % at most (Arntfield *et al*., 1997; Cenkowski & Sosulski, 1998; Arntfield *et al*., 2001; Phadi, 2004). The softening of texture during cooking of dry legume seeds has been attributed to the disintegration of the middle lamella between cotyledon parenchyma cells, protein denaturation and starch gelatinisation within the cotyledon parenchyma cells (Sefa-Dedeh & Stanley 1979a; Sefa-Dedeh *et al*., 1979). All these physicochemical and structural changes require the presence of water; hence improved water uptake during cooking of moisture-conditioned micronised legumes such as split peas, lentils and cowpeas has been related with reduction in cooking time (Cenkowski & Sosulski 1997; Arntfield *et al*., 2001; Phadi, 2004).

Improved hydration during cooking observed in moisture-conditioned and micronised legume seeds (Cenkowski & Sosulski, 1998; Fasina *et al*., 2001) has been attributed to the formation of a more open structure (Arntfield *et al*., 2001) and development of cracks, which facilitate the movement of water into the seeds. Arntfield *et al*. (2001) reported that moisture-conditioned and micronised lentils had an open structure that was more evident in intercellular spaces. This change in structure was attributed to the rapid evaporation of water from the cells during the micronisation process (Arntfield *et al*., 2001). Micronisation of biological material causes the water molecules to vibrate resulting in rapid internal heating and rise in water vapour pressure inside the material. This causes the seeds to increase in volume and lose moisture resulting in reduced density (Fasina *et al*., 2001) and sometimes popping (Phadi, 2004).

However, it has also been shown that improvement in hydration rate alone does not result in the desired shorter cooking time for micronised legume seeds (Abdul-Kadir *et al*., 1990). Abdul-Kadir *et al*. (1990) reported a 50 % increase in water uptake during soaking of infrared-heated (17 % moisture, 99 and 107 °C) black beans (*Phaseolus vulgaris*) that was accompanied by a 16 to 64 % increase in cooking time.

Similarly, Sarantinos and Black (1996) reported that when micronised (17 % moisture, 69, 88, 90 °C) chickpeas were soaked for 18 h and pressure-cooked, there was 15 -19 % increase in work required to compress the micronised samples as compared to untreated samples. These reports indicate that there is a possible hardening that occurs when legume seeds are micronised with limited moisture.

2.6.1.1 Effect of micronisation on the middle lamella in the cotyledon of legume seeds

Solubilisation of the middle lamella is one of the factors that contribute towards the softening of texture during the cooking of dry cowpea seeds (Sefa-Dedeh & Stanley, 1979a). Evidence on the role of the middle lamella in softening of cooked legume seeds has been reported in the form of SEM and pectin solubility. During the preparation of samples for SEM, fracture tends to occur along the weaker points. Sefa-Dedeh *et al*. (1978) reported that in raw legume seed such as cowpeas, the fracture line occurs across the cell, exposing the cross section of the cell and it's surrounding since the middle lamella is intact and strong. However, in samples that have been exposed to a type of hydrothermal treatment, the middle lamella is reportedly weaker due to β elimination of pectic substances (Liu *et al*., 1993b). Since the middle lamella is weakened, the parenchyma cells separate along the cell wall following the application of pressure (Sefa-Dedeh *et al*., 1978; Liu *et al*., 1993a). Arntfield *et al*. (2001) reported that cotyledon cells of micronised (33 % moisture, 138 ºC) lentils separated along the cell wall upon fracture during sample preparation for SEM, an indication of middle lamella disintegration. In addition, Arntfield *et al*. (1997) reported a significant reduction in pectic substances for micronised (29 % moisture, 88 °C) lentils. Thus micronisation of moisture-conditioned legumes might result in disintegration of the middle lamella, contributing towards shorter cooking time.

2.6.1.2 Effect of micronisation on physicochemical properties of starch in treated legume seeds

Gelatinisation of starch during cooking of legumes is another important phenomenon that has a positive correlation with texture of cooked seeds (Arntfield *et al*., 2001). Reports have shown that micronisation of moisture-conditioned seeds increases the level of enzyme-susceptible starch in treated legumes (Arntfield *et al*., 1997; Bellido

et al., 2006). Increased starch susceptibility to α-amylase digestion is generally used as an indication of starch gelatinisation, thus micronisation has been shown to pre-gelatinise starch in treated legumes (Arntfield *et al*., 1997; Bellido *et al*., 2006). Using SEM, Phadi (2004) observed that the shape of starch granules in micronised (41 % moisture, 140 - 160 °C) cowpea seeds was deformed possibly due to swelling during starch gelatinisation. Since the starch in micronised legume seeds (moisture-conditioned) is possibly pregelatinised, then moisture-conditioned and micronised seeds would require less time and energy to be cooked and have a softer texture during subsequent cooking. Cenkowski and Sosulski (1997) used differential scanning calorimetry (DSC) of lentil flours, to demonstrate that 5 min of cooking was required to adequately gelatinise the starch in micronised (38.6 % moisture, 150 °C) lentils as compared to 50 % of the starch for untreated lentils. In addition, a negative correlation has been reported between percent enzyme-susceptible starch and hardness of moisture-conditioned and micronised seeds during subsequent cooking (Arntfield *et al*., 1997; Bellido *et al*., 2006). The level of enzyme-susceptible starch in moisture-conditioned and micronised legume seeds has been positively related to moisture content during the micronisation process and moisture content of the micronised seeds (Arntfield *et al*., 1997). Arntfield *et al*. (1997) reported a significant (~50 %) increase in gelatinised starch (enzyme-susceptible starch) when the moisture content of lentils during micronisation was increased from 25 % to 33 %. Since gelatinisation of starch is a hydrothermal process, increasing the level of available moisture would result in increasing the level of starch gelatinisation.

Arntfield *et al*. (1997) reported that when preconditioned lentils (25, 29, 33 % moisture) were micronised (115 volts, 150 s) followed by air drying (room temperature) to three moisture levels (7, 9, 12 %), the amount of enzyme-susceptible starch decreased with the decreasing end moisture content. The reduction in enzyme-susceptible starch was possibly due to amylose association with amylose, amylopectin and lipids (Hoover & Manuel, 1996a). Gelatinisation of starch is the beginning of a process that continues with retrogradation especially in legume starches, which have higher amylose content. Following micronisation of moisture-conditioned legume seeds, amylose chains, solubilised during gelatinisation, re-associate to form crystalline double helices stabilised by hydrogen bonds (Jane and Robyt, 1984). Upon cooling and ageing, the helices aggregate to form three-dimensional crystalline

structures of the B-type. The amylose-amylose crystallites are highly stable with a melting endotherm at about 150 ºC and are resistant to enzyme digestion. This means that amylose-amylose crystallites would remain intact during normal cooking.

Arntfield *et al*. (2001) reported that there was no significant difference in the amount of enzyme-susceptible starch between lentils (33 % moisture) micronised to a high temperature (170 °C) and those micronised to a lower temperature (138 °C). However, when the two samples were cooked for 15 min, the lentils micronised to 170 °C were harder than the lentils micronised to 138 °C. It is apparent that enzyme-susceptible starch does not fully explain the contribution of starch gelatinisation in the micronisation-induced reduction of cooking time. Gelatinisation of starch is a complex process with different stages and products. Measurement of enzyme-susceptible starch indicates that there has been granular disorganisation to allow for enzyme digestion, but it does not show the extent of modification in the functionality of the starch in terms of its pasting properties (swelling and changing from glassy to rubbery state).

2.6.1.3 Effect of micronisation on physicochemical and functional properties of protein in treated legumes

Micronisation does not affect the total nitrogen content of legumes (Zheng, Fasina, Sosulski & Tyler, 1998). However, the protein has been reported to be denatured. In literature, the reported surface temperature of seeds during micronisation are generally greater than 90 °C, which is above the denaturation temperature for most plant storage proteins including cowpeas (Horax *et al*., 2004a). Protein denaturation refers to the loss of the native conformation due to changes in the stabilising effects of non covalent bonds (hydrophobic interactions, hydrogen and electrostatic bonds) and disulphide bonds (Damodaran, 1996a). Heat-induced denaturation of protein may vary from aggregation of polypeptide chains due to the formation of hydrophobic and disulphide bonds to pyrolysis depending on moisture content, heating temperature and time of exposure (Zheng *et al*., 1998).

Protein denaturation has been studied by monitoring physicochemical properties such as nitrogen solubility. Researchers (Arntfield *et al*., 1997; Cenkowski & Sosulski, 1998; Arntfield *et al*., 2001; Fasina *et al*., 2001; Bellido *et al*., 2006) on micronisation

of leguminous seeds (lentils, split peas, kidney beans, green peas, black beans, and pinto beans) have reported significant reduction in nitrogen solubility. Micronisation (18 % moisture, 140 °C) has been shown to reduce the solubility of albumin and globulin fractions (pH 6.0) of some legumes (green peas, yellow peas, kidney bean, black bean, lentil and pinto bean) by 12 - 41 % and 9 - 64 %, respectively (Zheng *et al*., 1998). Enwere, McWatters & Phillips (1998) reported that cowpea albumins were more heat labile and were denatured during hot air drying of cowpea seeds (34 % moisture) at elevated temperatures (80 - 120 °C). However, this was in contradiction with the results reported by Sefa-Dedeh & Stanley (1979c) who reported that water extractable cowpea proteins (albumins) were not coagulated by elevated temperatures. These differences on which fraction of cowpea protein is more susceptible to thermal denaturation could be due to differences in extraction conditions as well as the pH at which the solubility was being measured.

Reduction in nitrogen solubility of thermally treated legume seeds may result from the unfolding of protein molecules to expose hydrophobic sites leading to reduction in solubility (Zheng *et al*., 1998). Zheng *et al*. (1998) reported that the use of sodium dodecyl sulphate (SDS) in pH 10 borate buffer resulted in an improvement in the solubility of micronised legume seed albumin and globulin fractions. Addition of mercaptoethanol (MCE) to the SDS did not lead to further increase in protein solubility. This led to the conclusion that protein denaturation in micronised legumes was mainly due to hydrophobic aggregation rather than disulphide linkages (Zheng *et al*., 1998). However, this does not preclude the formation of other possible forms of intermolecular cross links which could lead to the formation of larger molecules with reduced water solubility. A number of crosslinks have been reported during thermal processing of protein-rich foods, which include disulphide, Maillard, isopeptide and dityrosyl crosslinks (Figure 2.4) (Gerrard, 2002).

Although cowpea protein is reported to be poor in sulphur containing amino acids (Chan & Phillips, 1994; Abu *et al*., 2005), intermolecular and intramolecular disulphide bonds have been reported in cowpea α-vignin and γ-vignin, respectively (Freitas *et al*., 2004). Intramolecular disulphide bonds are a result of protein folding that allows two cysteine residues to come into close proximity for oxidation of the sulphydryl groups to form a disulphide bond.

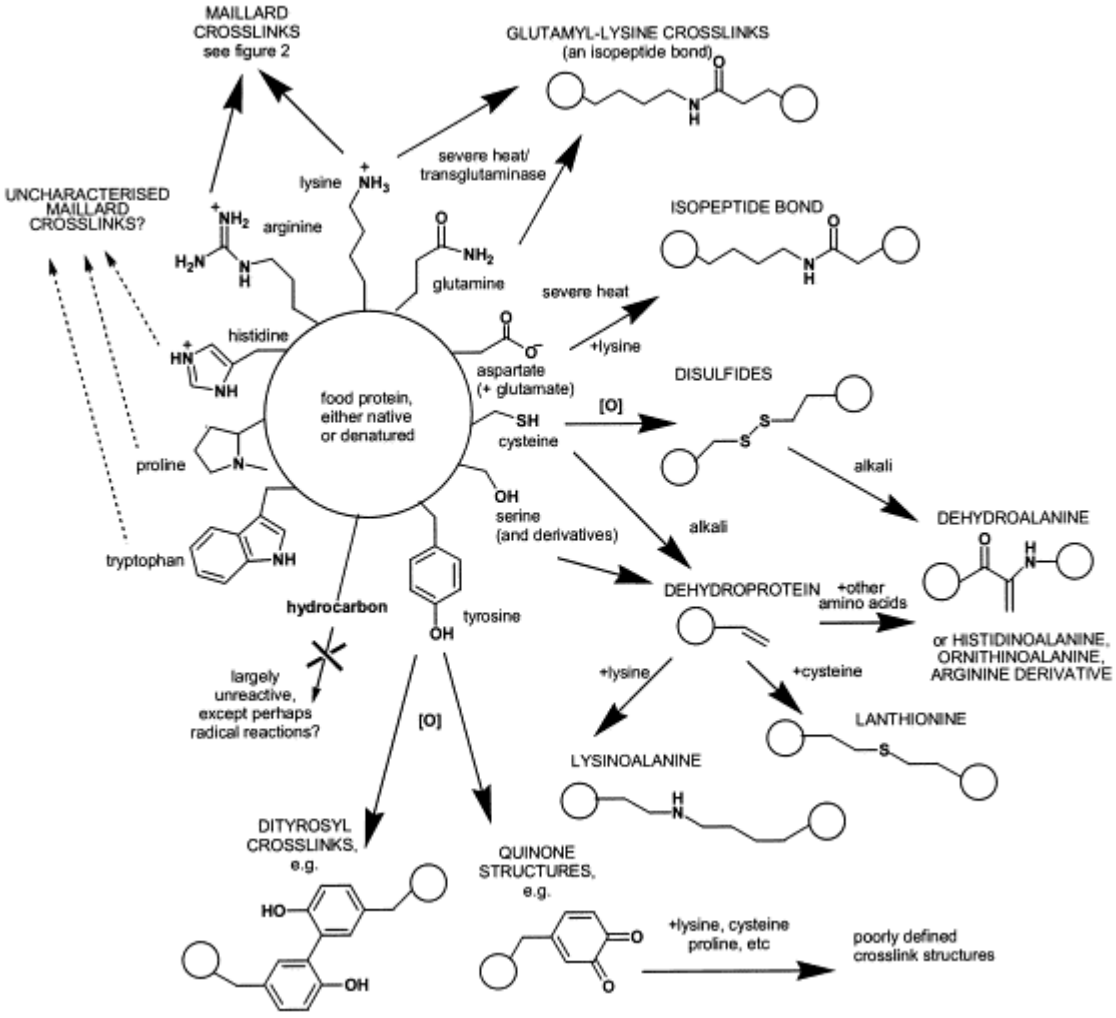

Figure 2.4 **A summary of crosslinking reactions that can occur during food processing** (Gerrard, 2002)

Non enzymatic browning has been suggested in micronised legume seeds especially where higher temperatures of about 170 and 180 °C were used (Arntfield *et al*., 2001; Phadi, 2004). Browning of seeds during micronisation of moisture-conditioned legume seeds could be attributed to Maillard reactions. Maillard reactions refers to a series of complex reactions that occur during processing at elevated temperatures which starts with a reaction involving amine and carbonyl groups and culminates with the formation of brown products known as melanoidins. In addition, dicarbonyl compounds formed during the browning process participate in crosslinking of proteins resulting in reduced protein solubility (Damodaran, 1996a; Gerrard & Brown, 2002).

Dityrosyl crosslinks involving two or three tyrosine residues have been reported in native glycoproteins in plant cell walls and wheat (Tilley, Benjamin, Bagorogoza, Okot-Kotber, Prakash & Kwen, 2001). In addition, exposure of tyrosine to peroxidase and hydrogen peroxide results in oxidation of tyrosine to dityrosine. Cowpea protein has tyrosine which could be oxidised to form dityrosyl cross links during thermal processing. Formation of dityrosyl cross links have also been reported in other forms of processing such as irradiation (Mezgheni, D'Aprano & Lacroix, 1998).

Under severe heat treatment, isopeptide cross links would possibly be formed, especially in foods of high protein and low carbohydrate content (Singh, 1991). Since the protein in cowpeas forms part of the cytoplasmic matrix and is also present as protein bodies, isopeptide crosslinks could be formed during micronisation, especially for the protein contained in protein bodies. Isopeptide cross links are formed by the condensation of the ε-amino group of a lysine residue with the amide group of an asparagine or glutamine residue. It has been suggested that the formation of isopeptide cross links increases with severity of a heat treatment (Singh, 1991).

Thermal denaturation of protein during micronisation has been reported to vary with severity of the treatment depending on moisture content, micronisation temperature and seed size. Overall, moisture affects thermal denaturation of proteins (Damodaran, 1996a) in that high moisture content during micronisation has been related to a decrease in protein solubility (Arntfield *et al*., 1997). On the contrary, Arntfield *et al*. (2001) reported that increasing the micronisation temperature from 138 to 170 °C did not result in significant reduction in protein solubility. This would possibly mean that increasing the micronisation temperature did not increase the amount of exposed hydrophobic sites or cross linking in order to further reduce the solubility of the protein nor was it severe enough for pyrolysis.

In addition, Fasina *et al*. (2001) reported that larger seeded legumes such as kidney and pinto beans had lower reduction in protein solubility than smaller seeded legumes (lentils and green peas) possibly due to the influence of seed size on the amount of IR penetrating the seeds. This would mean that during micronisation of small seeded legume seeds there is uniform treatment as compared to large seeded legumes where

the treatment would be more effective on the outer surfaces and not in the inside (Sarantinos & Black, 1996).

In summary, the existing work on micronisation and legume protein indicate that when legume seeds were micronised with limited moisture condition, there was reduction in protein solubility (Fasina *et al*., 2001), which was associated with increase in cooking time (Abdul-Kadir *et al*., 1990; Sarantinos & Black, 1996). Conversely, when micronisation (138 °C) was conducted with adequate moisture (33 % moisture) in the seed, the reduction in protein solubility was accompanied with a 63 % reduction in cooking time. On the contrary, when legume seeds with adequate moisture (33 % moisture) were micronised to high temperatures (170 °C) the reduction in protein solubility was accompanied with a 55 % reduction in cooking time. The increase in cooking time of legume seeds micronised with limited moisture results in protein denaturation without pregelatinisation of starch. Fasina *et al*. (2001) reported a less than 10 % increase in enzyme-susceptible starch for micronised (<10 % moisture) legumes, such that during subsequent cooking there is limited starch gelatinisation since the starch might have been modified by the heat treatment and was still embedded in the denatured protein matrix.

2.6.2 Effect of dry heat on functional properties of flour milled from treated legume seeds

The flour from moisture-conditioned and micronised cowpeas may have utilisation potential in some food systems depending on its functionality. There is limited information on the functionality of flour made from micronised legume seeds. However, reports have been made that micronisation reduces protein solubility in treated legume seeds; hence it would be expected to have a negative impact on protein-related functional properties of cowpea flour. Sarantinos and Black (1996) reported that micronisation (17 % moisture, 69 to 90 °C) did not have a definite effect on oil absorption capacity of chick pea flour. This concurs with the observation made by Prinyawiwatkul *et al*. (1997a) that oil absorption capacity of cowpea flour did not change with most processing methods.

Enwere *et al*. (1998) reported that thermal (hot air) treatment of cowpea seeds (34 % moisture, 120 °C) resulted in the loss of foaming capacity for cowpea flour. The loss

in foaming capacity of the flour from heat treated cowpeas was attributed to the denaturation of the albumin fraction of cowpea protein (Enwere *et al*., 1998). Thus flour from micronised legumes may not be suitable for foam textured products such as *akara*.

Sarantinos & Black (1996) reported that micronisation (69 to 90 °C) of chick pea seeds (17 % moisture) reduced the water absorption capacity of the flour milled from treated seeds. On the contrary, Fasina *et al*. (2001) reported that micronisation (< 10 % moisture, 140 °C) improved the water absorption capacity of a number of legume seeds (kidney beans, green peas, black beans, lentils & pinto beans). The improved hydration capacity for the flour was attributed to protein gelation and the negligible increase in enzyme-susceptible starch. Increase in water holding capacity of flour milled from hydrothermally treated cowpeas has also been reported (Phillips, Chinnan, Branch, Miller & McWatters, 1988; Prinyawiwatkul *et al*. 1997a) suggesting that the water holding capacity of thermally treated protein was affected by protein content rather than protein solubility. Phillips *et al*. (1988) observed that mild thermal treatment (25 % moisture, 70 °C, 80.5 min) resulted in increased water holding capacity which decreased with severe heat treatment (25 % moisture, 130 °C, 25 min). The increase in water holding capacity could possibly be due to protein denaturation leading to gelation. When the denatured protein is rehydrated, the water is physically entrapped in the gel, thus increasing the hydration capacity (Damodaran, 1996b).

Pasting properties are an important functional property for flour since they have an effect on viscosity and texture of food systems in which the flour is used. Cold swelling systems have not been reported in flour from micronised legume seeds although the starch is believed to be pregelatinised. Cenkowski and Sosulski (1998) reported that micronisation did not change the gelatinisation temperature of flour from micronised (26 % moisture, 120 °C) peas although there was significant increase in pasting viscosities throughout the heating and cooling processes. Similarly, Fasina *et al*. (2001) reported increased pasting viscosities of legume (kidney, pinto, and black beans, and green peas) flour following micronisation (< 10 % moisture, 140 °C). Cenkowski and Sosulski (1998) attributed the increase in pasting viscosity to structural changes in the flour from micronised (26 % moisture, 120 °C) seeds. Prior to heating (50 °C), SEM micrographs of the untreated flour dispersion exhibited fewer

starch granules among heterogeneous cellular contents while in the micronised (26 % moisture, 120 °C) slurry the starch granules were coated with a protein material and unattached to other cellular components. Since the granules in micronised (26 % moisture, 120 °C) peas were free, they swelled more during heating hence the increase in viscosity, which was accompanied by increase in amylose leaching resulting in higher set back viscosity than the control. Enwere *et al*. (1998) also reported that starch granules in cowpea flour from hot air dried seeds (34 % moisture, 120 °C) were dislodged from the protein matrix during fracture.

2.7 Gaps in knowledge

Micronisation of moisture-conditioned legume seeds (common beans, lentils, split peas and cowpeas) has been shown to result in structural and physicochemical changes that result in reduced cooking time. Reports (Arntfield *et al*., 2001; Phadi, 2004) indicate that the effectiveness of the process in reducing cooking time of moisture conditioned seeds is reduced when very high temperatures (>160 °C) are used. There is lack of information on the possible mechanism with which micronisation of moisture-conditioned legume seeds reduces cooking time. Hence it is not clear as to what changes occur in moisture-conditioned seeds micronised to higher temperatures that negatively impacts on the effectiveness of the process. Various researchers have reported on different aspects that may contribute towards the reduction in cooking time.

It has been recognised that micronisation improves the hydration rate of seeds which has been attributed to increased volume, reduced seed density, cracking and starch gelatinisation. However, there are contradictory reports on the effect of micronisation on legume seed coat. Abdul Kadir *et al*. (1991) suggested that micronisation may have caused cracks in the seed coat but not the cotyledon; on the contrary Arntfield *et al*. (2001) observed that micronisation did not change the structure of the seed coat in laird lentils. An open cotyledon structure has been suggested by the latter in moisture-conditioned and micronised lentil seeds although there is no concrete evidence by these researchers on whether the open structure is within the parenchyma cells, between cells or on the cotyledon surface.

Starch in moisture-conditioned and micronised legume seeds has been found to be more susceptible to α-amylase digestion (Arntfield *et al*., 1997; Arntfield *et al*., 2001) and has reduced thermal transition of the starch in unmicronised seeds (Cenkowski & Sosulski, 1998). However, this apparent starch granular modification (in terms of structure) was not adequate to allow for swelling at temperatures below the gelatinisation temperature (Cenkowski & Sosulski, 1998). It is therefore questionable whether this starch in moisture-conditioned and micronised legumes greatly contributes towards improved hydration properties during soaking of whole seeds and hydration capacity of the flour as has been suggested by various researchers (Cenkowski & Sosulski, 1998). Therefore, there is a need to further explore starch structural changes during micronisation at low and high temperatures.

Micronisation has been reported to reduce the solubility of legume seed proteins due to increased hydrophobic interactions (Zheng *et al*., 1998) as a result of thermally induced molecular unfolding of the protein. However, micronisation may also result in thermally induced cross linking such as Maillard, isopeptide, dityrosyl and disulphide. The formation of Maillard and isopeptide cross links may depend on micronisation temperatures involved in the process and it still remains to be determined if variation in micronising temperature would result in similar forms of protein denaturation. Additionally there is lack of information on the effect of micronisation of moisture-conditioned legumes at low and high temperatures on the behaviour of starch granules and protein matrix during subsequent cooking.

Literature has indicated that splitting of legume seeds during cooking is an undesirable characteristic for a whole seeded product (Taiwo *et al*., 1997a; Afoakwa *et al*., 2006). There is lack of information on the underlying physicochemical and structural properties that cause splitting during cooking of cowpeas. In addition, since micronisation may cause cracks in the legume seed; this could affect the integrity of whole seeds during subsequent cooking. No information is available in the literature on this phenomenon in micronised legume seeds.

Most of the research on micronisation has investigated micronisation of whole or split legume seed products and its effect on cooking time. However, information on the functionality of flour from micronised legume seeds is non existent.

2.8 Hypotheses

When different cowpea varieties are micronised (41 % moisture, 153 °C), their cooking characteristics (cooking time, texture and splitting) will differ due to inherent differences in seed structure (seed coat and cotyledon) and physicochemical characteristics (density, protein, starch).

If cowpeas (41 % moisture) are micronised to a low (130 °C) final surface temperature, then the treated cowpeas will have a shorter cooking time and a softer cooked texture during subsequent cooking than unmicronised cowpeas due to fissures in the seed coat and cotyledon (which improve water absorption during soaking and cooking), disintegration of the middle lamella, protein denaturation and partial starch gelatinisation. However, when higher (170 °C) final surface micronisation temperature is used, then the treatment will be less effective in reducing cooking time of cowpeas possibly due to protein-protein, protein-carbohydrate cross-linking and amylose associations in the cotyledon.

Micronisation of moisture-conditioned cowpea seeds will pregelatinise starch leading to amylose associations and will also denature the proteins through molecular unfolding, formation of disulphide, dityrosyl and isopeptide bonds as well as Maillard browning to different degrees depending on the surface temperature attained. These physicochemical changes will result in the modification of the functional properties of the flour milled from the moisture-conditioned and micronised cowpeas.

2.9 Objectives

The primary objective of this research was to determine the effect (s) of hydrothermal treatment (micronisation of moisture-conditioned seeds), on physicochemical and structural properties of cowpea seeds as they relate to cooking quality characteristics of whole seeds and functional properties of the resultant flours.

The specific objectives were to:

1. Determine the effect of hydrothermal (tempering and micronisation 153 °C) treatment on the cooking characteristics and seed microstructure of two cowpea varieties.

2. Determine the effect (s) of low (130 °C) and high (170 °C) micronisation temperatures on cooking characteristics and seed microstructure of conditioned Bechuana white cowpeas.
3. Determine the effect (s) of low (130 °C) and high (170 °C) micronisation temperatures on structural and physicochemical properties of starch and protein in micronised (41 % moisture, 130 and 170 °C) cowpea seeds.
4. Determine the effect (s) of low (130 °C) and high (170 °C) micronisation temperatures on functional properties of flour milled from micronised (41 % moisture, 130 and 170 °C) Bechuana white cowpea seeds.