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Physicochemical characteristics of conditioned and micronised cowpeas and functional properties of the resultant flours

By

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DEDICATION

This thesis is dedicated to the memory of my beloved mother, late Dorothy Irene Mvula

and

To the Lord my God, my ever present help in times of need;

I lift my eyes to the hills

where does my help come from

my help comes from the Lord

maker of heaven and earth.

DECLARATION

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

Agnes Mbachi Mwangwela
2006

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ABSTRACT

Physicochemical characteristics of conditioned and micronised cowpeas and functional properties of the resultant flours

By

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Promoter: Professor Amanda Minnaar
Co-promoter: Professor Ralph D. Waniska (Texas A & M University)
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Cowpea (*Vigna unguiculata* L. Walp) is an important source of protein in some parts of sub Saharan Africa. In southern Africa, it is mainly boiled into a stew, and long cooking time is a concern. Micronisation of preconditioned seeds has been used to reduce the cooking time of other dry legume seeds such as lentils. Hence micronisation (moisture conditioning and infrared heating) presents an opportunity for processing cowpeas to alleviate long cooking time and provide a convenient product as well as diversify cowpea products. In addition, potential exists for using flour milled from micronised (moisture conditioned and infrared heated) seeds in food systems. However, variations in raw material physicochemical properties (seeds) and micronisation temperature would affect the efficacy of the process to produce products with desired properties. Mild (130 and 153 °C) and severe (170 °C) final surface temperatures were used to determine the extent of micronisation-induced changes in cowpea structure and physicochemical properties and functionality of the resultant flours.

Two cowpea varieties (Bechuana white and Var. 462; 41 % moisture) micronised to 153 °C were used to study the effect of micronisation on physicochemical and structural properties of cowpea seeds. Bechuana white (41 % moisture) micronised to three

temperatures (130, 153 and 170 °C) was used to study the micronisation temperature effect on physicochemical properties of cowpeas and functional properties of resultant flours. Scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) were used to study seed structure, while light microscopy was used for the flour. Gel permeation high performance liquid chromatography (GP-HPLC), differential scanning calorimetry (DSC), and a rapid visco-analyser (RVA) were used to study starch-related properties, while fluorescence spectroscopy and electrophoresis (SDS-PAGE) were used to study physicochemical properties of isolated proteins. These physicochemical and structural properties were determined to aid in explaining the possible micronisation-induced changes in cooking characteristics of seeds and functional properties of the resultant flours.

Micronisation (41 % moisture, 153 °C) reduced cooking time (Bechuana white > Var. 462) and increased splitting (Var. 462 >Bechuana white) of the cowpeas during subsequent cooking. The micronised (41 % moisture, 153 °C) seeds were relatively softer than the unmicronised samples following subsequent cooking. The mild temperatures (130, 153 °C) were more effective in reducing cooking time than the higher temperature (170 °C). Micronisation (41 % moisture, 153 and 170 °C) caused physical fissuring of the seed coat, cotyledon, and parenchyma cell wall and reduced the bulk density of treated seeds. These changes in the physical structure improved the hydration rate of the seeds during cooking. There is a possibility that micronisation (41 % moisture, 130, 153, 170 °C) also caused the degradation of pectic substances of the middle lamella, since shorter cooking time was required for cotyledon parenchyma cells to separate during cooking.

Mild micronisation (41 % moisture, 130 and 153 °C) temperatures caused the disruption of the native starch granular order leading to retrogradation of amylose, while at higher micronisation (41 % moisture, 170 °C) temperatures the retrogradation of amylose was possibly accompanied by endodegradation of starch. Simultaneously, micronisation (41 % moisture, 130 and 170 °C) led to increased surface hydrophobicity and crosslinking of protein, which was more pronounced in M-170 °C samples. SDS-PAGE

indicated that disulphide bonds were formed in micronised (41 % moisture, 130 and 170 °C) samples; while isopeptide bonds, dityrosyl bonds and Maillard derived crosslinks are possibilities especially in the M-170 °C sample. The pronounced crosslinking of protein and possible depolymerisation of starch contributed to hardening of the cotyledon structure, consequently impacting negatively on the effectiveness of the micronisation (41 % moisture, 170°C) treatment in reducing cooking time.

These changes in seed structure and physicochemical properties of starch and protein contributed towards the reduction in cooking time and increased splitting of seeds and modified flour functionality. Cowpea flour foaming capacity was lost following micronisation (41 % moisture, 130 and 170 °C) possibly due to reduced solubility and crosslinking of protein. Micronisation (41 % moisture, 130 and 170 °C) reduced flour gelling and pasting properties while increasing the water absorption capacity, more so in M-170 °C samples than in M-130°C. Hence micronisation to mild temperatures (130 °C) has the potential of producing cowpeas with shorter cooking time, which can also be milled into flour with modified functionality. Thus micronisation of moisture-conditioned cowpeas to mild temperatures would contribute towards increased utilisation of cowpeas as well as improving household nutrition status.

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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.

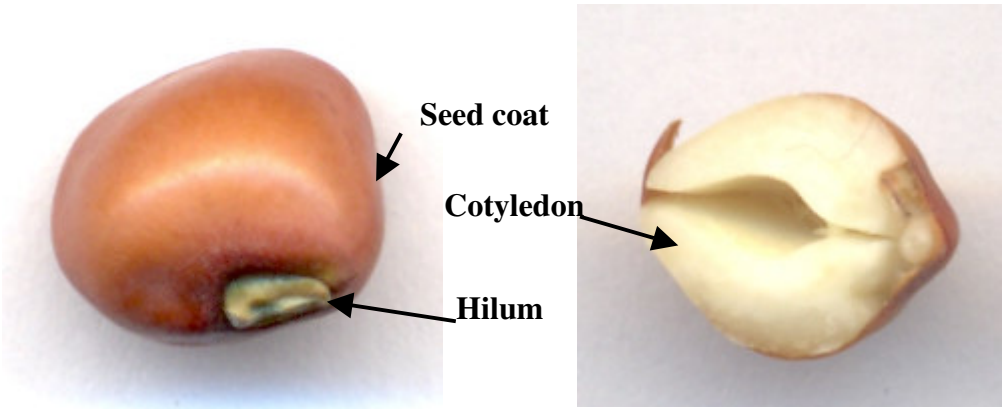

Figure 2.1 Morphology of the cowpea seed showing seed coat, hilum and cotyledon

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).

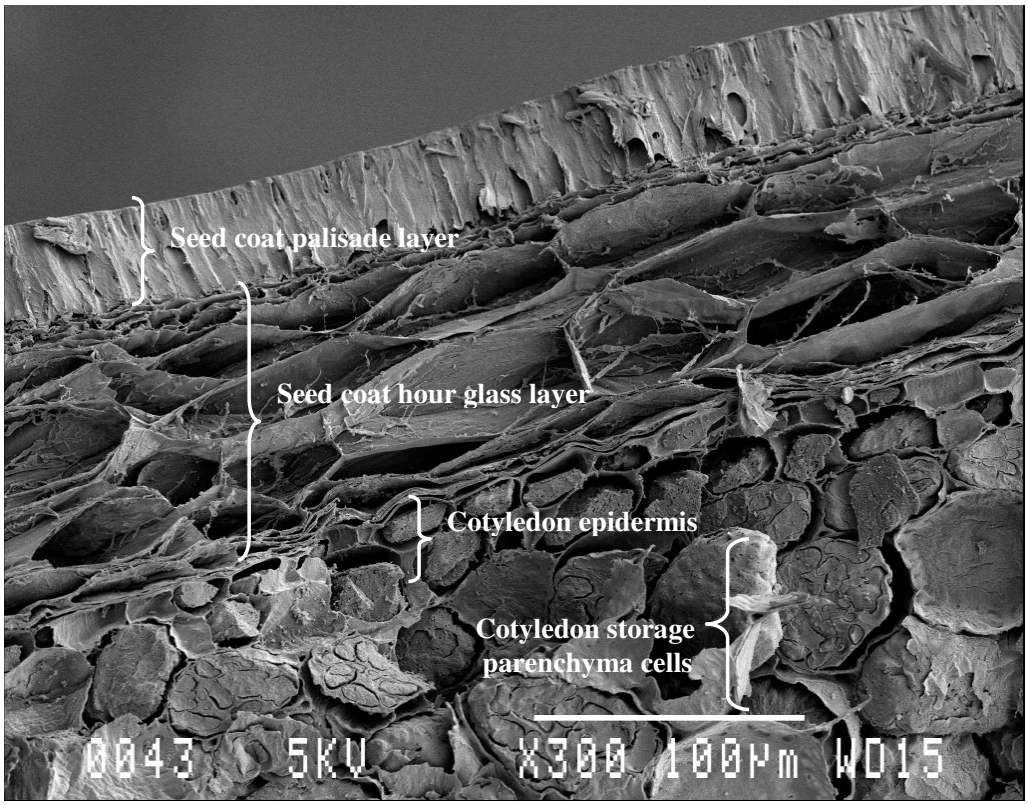

Figure 2.2 **Cross section of a dry Bechuana white cowpea (*Vigna unguiculata*) seed** (Phadi, 2004)

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).

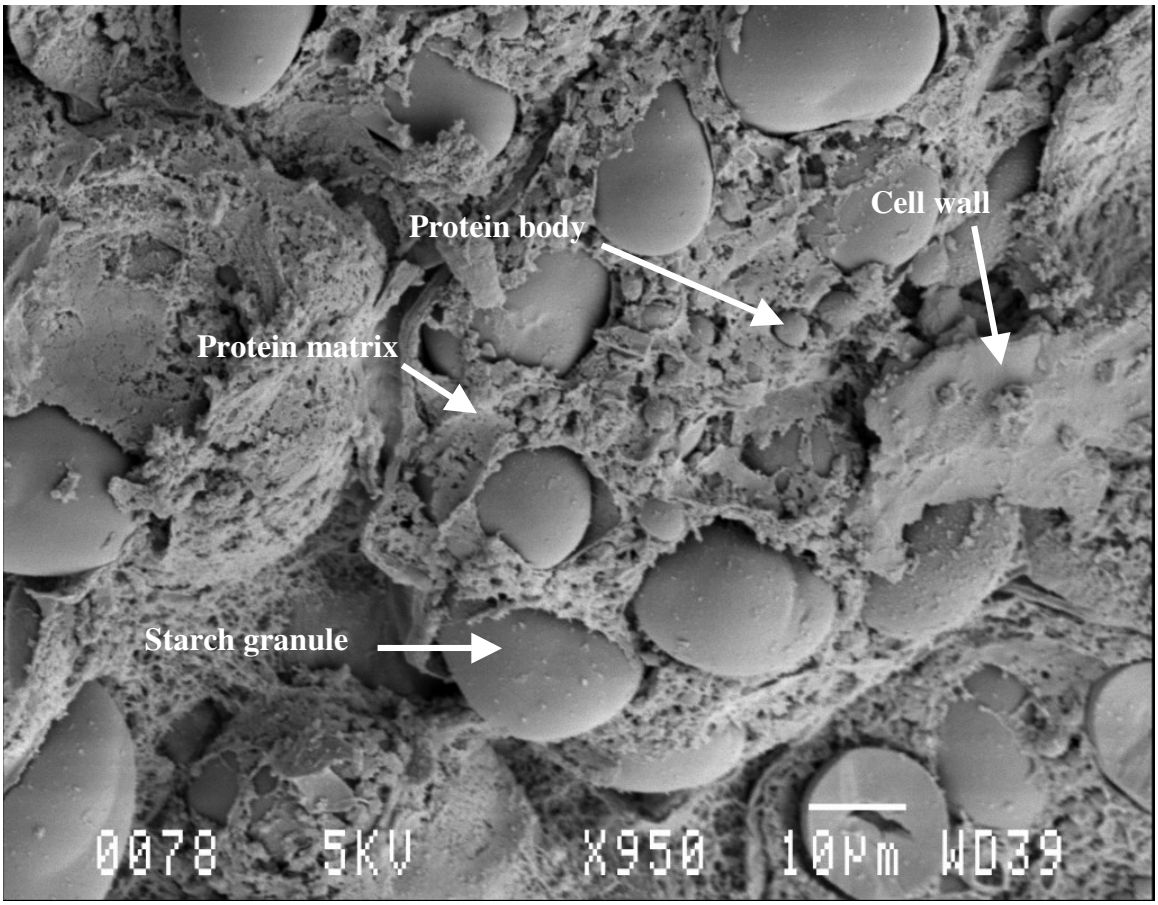

Figure 2.3 **Cross section of tempered (41 % moisture) Bechuana white cowpea seed cotyledon** (Phadi, 2004)

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.

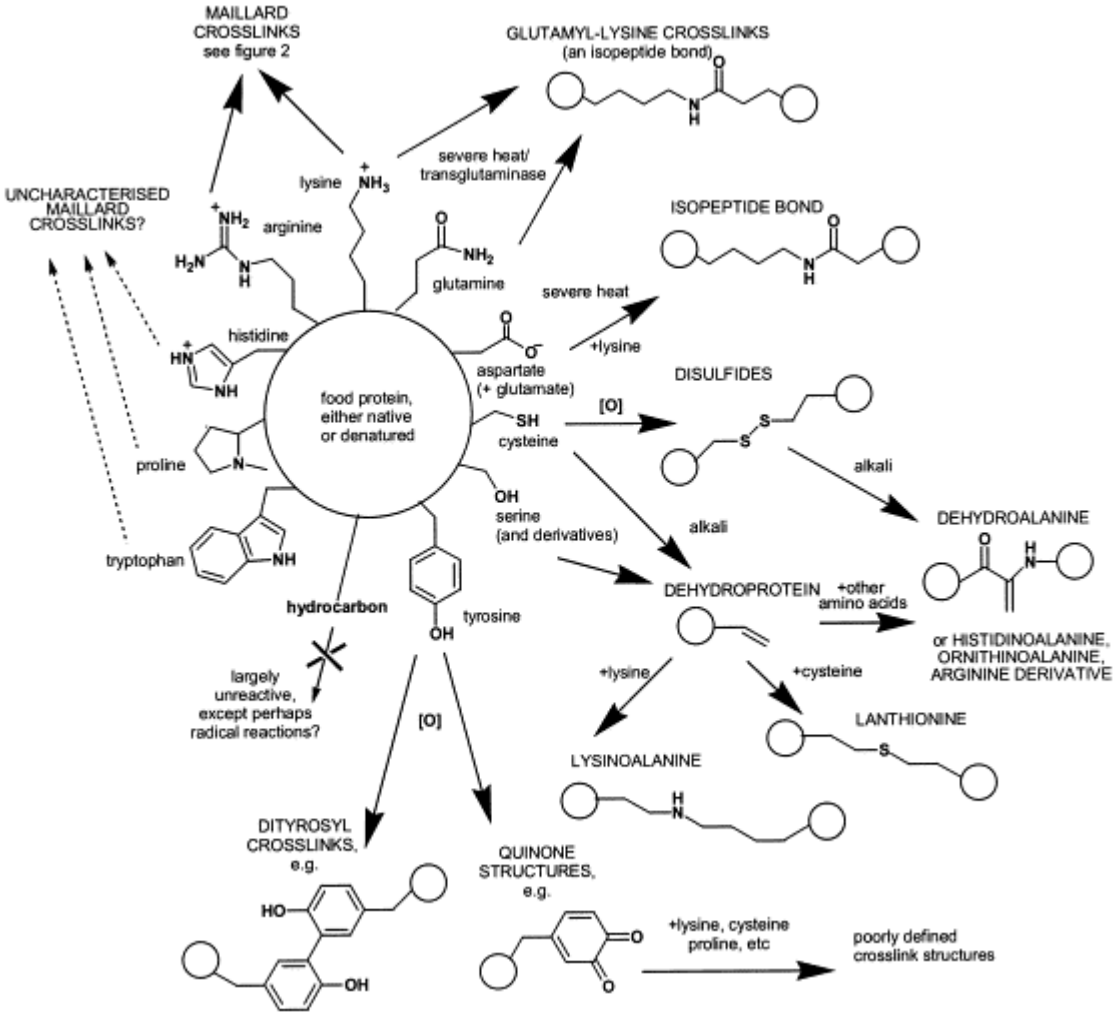

Figure 2.4 **A summary of crosslinking reactions that can occur during food processing** (Gerrard, 2002)

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.

3 RESEARCH

The research was conducted in three phases. Preliminary work, which is reported in section 3.1, was done to select two cowpea varieties from a collection of varieties obtained from Bunda College of Agriculture in Malawi and Agricol in South Africa. Once the two cowpea varieties were identified, the second phase investigating the effect of micronisation which comprised of tempering the cowpea seeds and infrared heating, on cooking characteristics of the selected cowpea varieties was done and this is reported in section 3.2. The third phase concentrated on the underlying structural and physicochemical changes that contributed to the change in cooking characteristics of the cowpea seeds and functionality of the resultant flours. The work from the third phase is reported in sections 3.3 and 3.4.

3.1 Physicochemical and cooking characterisation of nine cowpea (*Vigna unguiculata* L. Walp) varieties

Abstract

Nine cowpea (*Vigna unguiculata* L.Walp) varieties were characterised in order to identify two varieties with divergent cooking characteristics. The cowpeas were significantly ($P \leq 0.05$) different in size ranging from 11.4 g to 14.1 g per 100 seeds of Bechuana white and Var. D, respectively. Bechuana white and Agribleu had significantly ($P \leq 0.05$) lower rate of water uptake during soaking although the amount of water absorbed during cooking followed a similar trend for all the varieties. Var. 462 had a significantly ($P \leq 0.05$) harder texture than all the other cowpea varieties after 75 min of cooking, while Bechuana white had significantly ($P \leq 0.05$) higher incidence of splits during cooking than the other cowpeas. The greatest variation in cooked texture and splitting was observed between Var. 462 and Bechuana white, and these were selected for the main study.

Key words: cowpeas, physicochemical, cooking time, texture, splits, water absorption

3.1.1 Introduction

Cowpeas (*Vigna unguiculata* L. Walp) are widely produced and consumed in most developing countries of sub Saharan Africa where they are a good source of affordable protein and vitamins to the mainly carbohydrate-based diet of sub Saharan Africa (Phillips *et al*., 2003). The optimum utilisation of cowpeas however faces challenges such as long cooking time that is required for the cowpeas to attain soft texture and be palatable for consumption (Taiwo, 1998). Long cooking time of legumes such as cowpeas leads to increased energy use particularly in rural and peri urban areas where fuel wood is the main source of household energy (Brouwer *et al*., 1996). In addition, long cooking time means that women have to spend more time in meal preparation. The cooking time of cowpeas is mainly dependent on variety due to differences in physicochemical properties such as protein, calcium and phytic acid (Akinyele *et al*., 1986). Storage conditions of the seeds (Abu, Arogba & Ugwu, 1999) and ionic composition of the water used for cooking (Onigbinde & Ojeabulu, 1999) also have an effect on cooking time.

Several methods have been proposed and are used to reduce the cooking time of cowpeas. Soaking in water or dilute organic acids or sodium salt solutions (Onigbinde & Ojeabulu, 1999), have been used to reduce the cooking time of cowpeas. Micronisation has been used to process and significantly reduce the cooking time of legumes such as lentils (Arntfield *et al*., 1997). Micronisation refers to infrared heating, and has been applied in legumes such as split peas, lentils and black beans (Cenkowski & Sosulski, 1997; Cenkowski & Sosulski, 1998; Bellido *et al*., 2006). The application of micronisation as a precooking process for dried whole cowpeas will depend on the cooking characteristics of both the raw material and the processed product. The success of micronisation of whole cowpea products will depend on the quality characteristics of the moisture-conditioned and micronised products. Cooking quality in legumes encompasses several characteristics such as cooking time, splitting during cooking, texture and other sensory attributes.

The objective of this study was to characterise the cooking qualities of nine cowpea varieties from the region in order to identify the varieties with most divergent physicochemical characteristics (protein and moisture contents, water uptake during soaking and cooking, splitting of cooked cowpeas and cooked texture) in order to

select the two most divergent varieties, which could be used to determine the structural and physical chemical changes caused by micronisation of moisture-conditioned seeds.

3.1.2 Materials and methods

Seven cowpea varieties were obtained from Bunda College of Agriculture in Malawi and two from Agricol in Potchefstroom, South Africa. Cowpea samples were cleaned to remove chaff, shrivelled and broken seeds. The cleaned seeds were packed in polypropylene bags and stored at 4 °C until the time of use.

3.1.2.1 Moisture determination

Moisture content of the cowpea seeds was determined according to the method of Ajibola, Aviara and Ajetumobi (2003). Thirty grams (M0) of cowpea seeds were weighed into pre dried (1 h, 103 ºC) moisture tins that had been cooled in a dessicator. The samples were then dried for 72 h in a hot air oven at 103 ºC. The dried samples were then cooled in a dessicator and weighed (M1). Moisture content of the cowpea seeds expressed as g of water kg^{-1} cowpea was calculated as follows:

Moisture content = {(M0-M1)/M0} * 1000

3.1.2.2 Crude protein determination

Crude protein content of the cowpeas was determined using the Dumas method. A factor of 6.25 was used to calculate the crude protein from the nitrogen content determined using a Leco Nitrogen Analyser FP 528 (Leco Africa Pty, Kempton Park, South Africa).

3.1.2.3 Seed size determination

The size of cowpeas was expressed as the weight of 100 seeds weighed using a Precisa (0.01g) top loading balance.

3.1.2.4 Determination of water absorption during soaking

Water absorption during soaking was determined according to a modified method of Agbo, Hosfield, Uebersax, and Kimparens, (1987). Approximately 10 g of cowpea seeds were placed in 100 ml Erlenmeyer flasks containing 50 ml deionised water. The Erlenmeyer flasks were placed in an incubator at 22 °C for 1, 2, 3, 4, 5 and 6 h. After

soaking, the excess water was drained using a metal sieve (2.5 mm) and the cowpeas were blotted dry with absorbent paper to remove excess water and weighed. The gain in weight was expressed as g of water kg^{-1} cowpea.

3.1.2.5 Determination of water absorption during cooking

The amount of water absorbed during cooking was determined according to a modified method of Cenkowski and Sosulski (1997). For each cowpea variety approximately 10 g of cowpea seeds were placed in 100 ml Erlenmeyer flasks containing 50 ml deionised water. The Erlenmeyer flasks were placed in a heavy aluminium pan containing 1500 ml of deionised water. The pan was tightly covered and brought to boil, allowing 5 min for heating up. The cowpeas were cooked up to 90 min. Every 15 min, two sample flasks per cowpea variety were removed and excess water was drained using a metal sieve (2.5 mm). Cowpeas were cooled to room temperature (22 ± 2 °C) for 1 h, blotted dry with absorbent paper to remove excess water and weighed. The gain in weight (g) was expressed as g water kg^{-1} cowpea. The boiled cowpeas were then used to determine splitting and texture.

3.1.2.6 Determination of splitting during cooking of cowpea seeds

The tendency of seeds to split during cooking was determined according to the method of van Buren, Bourne, Downing, Quele, Chise, and Comstock (1996). The cowpeas with split seed coats and cotyledon were counted as splits. The degree of split was calculated as follows:

$$\frac{\text{Number of split seeds}}{\text{Number of whole seeds}} \times 100$$

3.1.2.7 Determination of seed texture during cooking of cowpea seeds

Texture was measured as work (area under the curve, Nmm) done to cut individual cowpea seeds through a distance of 5 mm at a speed of 4 mm/s based on the method of Sefa-Dedeh *et al*. (1978). A texture analyser (TA-XT2, Stable Micro Systems, Surrey, England) with A/CKB craft knife adapter mounted with Hilite® heavy-duty blades (Hilite Hardware, Hatfield, Pretoria, South Africa) was used to measure texture. A single cowpea seed was positioned on its side and cut below the hilum.

The blade was replaced after 5 determinations. Four seeds were measured per sample of cowpeas cooked for 15, 30, 45, 60, 75 and 90 min.

3.1.2.8 Statistical analysis

The experimental work was conducted on duplicate samples to generate data that was analysed using Statistica statistical software version 6.0. Analysis of variance (ANOVA) was used to evaluate the data based on a 5 % level of significance. When the F-test was significant, differences between means were determined using the Least Significant Difference (LSD) test.

3.1.3 Results and discussion

Nine cowpea varieties were sourced and described (Table 3.1.1). All the cowpeas used in the study had smooth seed coats. The moisture content of the cowpeas ranged from 89 to 121 g of water kg^{-1} of Bechuana white and Var. D, respectively. The crude protein content of the cowpeas ranged from 240 to 283 g of protein kg^{-1} and was within the range reported in literature. Akinyele *et al*. (1986) alluded to a possible effect of protein content on cooking time. A positive correlation between cooking time and protein content of cowpeas was reported (Akinyele *et al*., 1986).

Bechuana white and Var. 462 were relatively smaller in size when compared with the other varieties (Table 3.1.1). Demooy & Demooy (1990) reported that small seeded cowpeas have been reported to have longer cooking time as compared to the larger seeded varieties. However this is in contrast to results found by Olapade *et al*. (2002) where small seeded cowpeas had cooking times which were comparable to large seeded cowpeas. Var. 418 and Agribleu were relatively large seeded and would be preferable to consumers (Langyintuo *et al*., 2004). Olapade *et al*. (2002) reported the seed size of cowpeas to range from 1.92 to 24.4 g per 100 seeds. Hence the cowpeas used in this study would be medium sized. The small to medium sized cowpeas seeds would be suitable for micronisation in order to attain uniform heat transfer (Fasina *et al*., 2001). Cenkowski and Sosulski (1998) alluded to the difficulty of obtaining uniform heating through the depth of large seeds as compared to smaller seeds.

Table 3.1.1 Source and selected physicochemical characteristics of nine cowpea varieties

Variety	Source	Colour	Moisture (g kg^{-1})	Protein (g kg^{-1})	Seed size (g/100 seeds)	Texture (work) after 75 min of cooking (N mm)
Var. 223/1	Bunda College	Pinkish	113^{b} (6.9)	283^{e} (4.4)	13.9^{cd} (0.40)	4.10^{ab} (0.94)
Var. 418	Bunda College	Maroon	107^{b} (6.2)	260^{bc} (5.0)	15.0^{e} (0.48)	5.99^{bc} (0.64)
Bechuana white	Agricol- South Africa	Cream	89^{a} (3.8)	240^{a} (1.6)	11.4^{a} (0.14)	3.31^{a} (0.75)
Agribleu	Agricol- South Africa	Purple	109^{b} (8.2)	267^{c} (3.5)	14.7^{de} (0.45)	3.71^{ab} (0.52)
Var. 462	Bunda College	Maroon	11.7^{bc} (8.0)	257^{b} (2.4)	11.2^{a} (0.24)	7.34^{c} (3.74)
Var. A	Bunda College	Beige to brown	113^{b} (6.9)	280^{e} (4.2)	12.6^{b} (0.55)	4.56^{ab} (1.29)
Var. B	Bunda College	Beige to brown	107^{b}(10)	279^{e} (9.8)	13.1^{bc} (1.11)	5.58^{abc} (1.31)
Var. C	Bunda College	Beige to brown	120^{c} (1.4)	272^{d} (6.8)	14.1^{d} (0.95)	4.36^{ab} (0.46)
Var. D	Bunda College	Beige to brown	121^{c} (3.6)	266^{cd} (4.2)	13.4^{c} (0.52)	4.35^{ab} (0.96)

Means followed by the same superscript in a column are not significantly different at $P \leq 0.05$; Standard deviations of the means are in parenthesis

3.1.3.1 Water uptake during soaking

During traditional boiling of legumes such as cowpeas, soaking is used to enhance rehydration of the seed contributing to shorter cooking time. In all the cowpea varieties the amount of water absorbed per unit weight of the seeds increased with increasing soaking time (Figure 3.1.1), a pattern that has been reported in previous cowpea work (Taiwo *et al*., 1998). Var. 223/1 exhibited significantly ($P \leq 0.05$) higher initial (3 h) water absorption while Bechuana white had the lowest. High rate of water absorption during soaking has been related to amorphous and thin seed coats (Sefa-Dedeh & Stanley, 1979a). Bechuana white and Agribleu absorbed significantly ($P \leq 0.05$) lower amount of water per unit weight of cowpeas after 6 h of soaking than the other cowpea varieties.

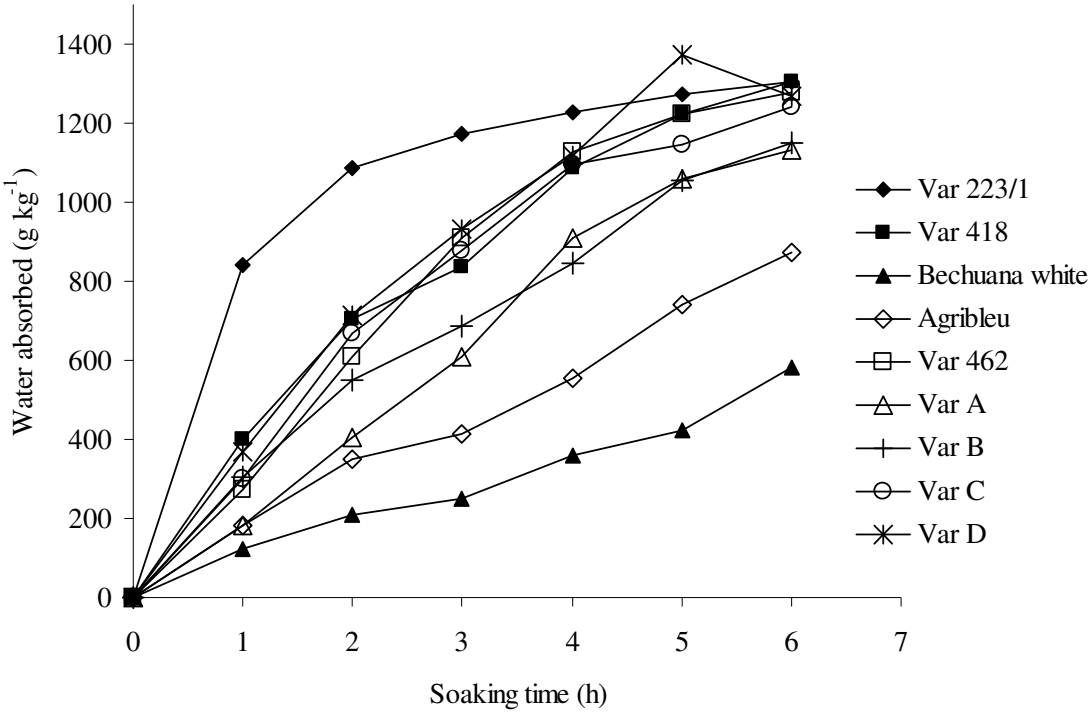

Figure 3.1.1 Water absorption patterns for 9 cowpea varieties during 6 h of soaking

The hydration properties of the cowpeas are important in determining the parameters considered during tempering of cowpeas prior to micronisation. Dry cowpeas can be

rehydrated either by steeping the seeds in water for a specific period of time or adding a predetermined amount of water to the sample in order to adjust the moisture content to targeted moisture content (Phadi, 2004). Tempering of cowpeas is an important step required to maximise the effect of micronisation for legumes such as cowpeas (Arntfield *et al*., 1997). Adequate rehydration is necessary for the gelatinisation of starch during the micronisation of pulses (Arntfield *et al*., 1997) such as cowpeas.

3.1.3.2 Water absorption during cooking

The amount of water absorbed by all the cowpea varieties increased with increase in cooking time (Figure 3.1.2). The increase in the amount of water absorbed by the cowpeas is possibly due to starch gelatinisation and protein gelation.

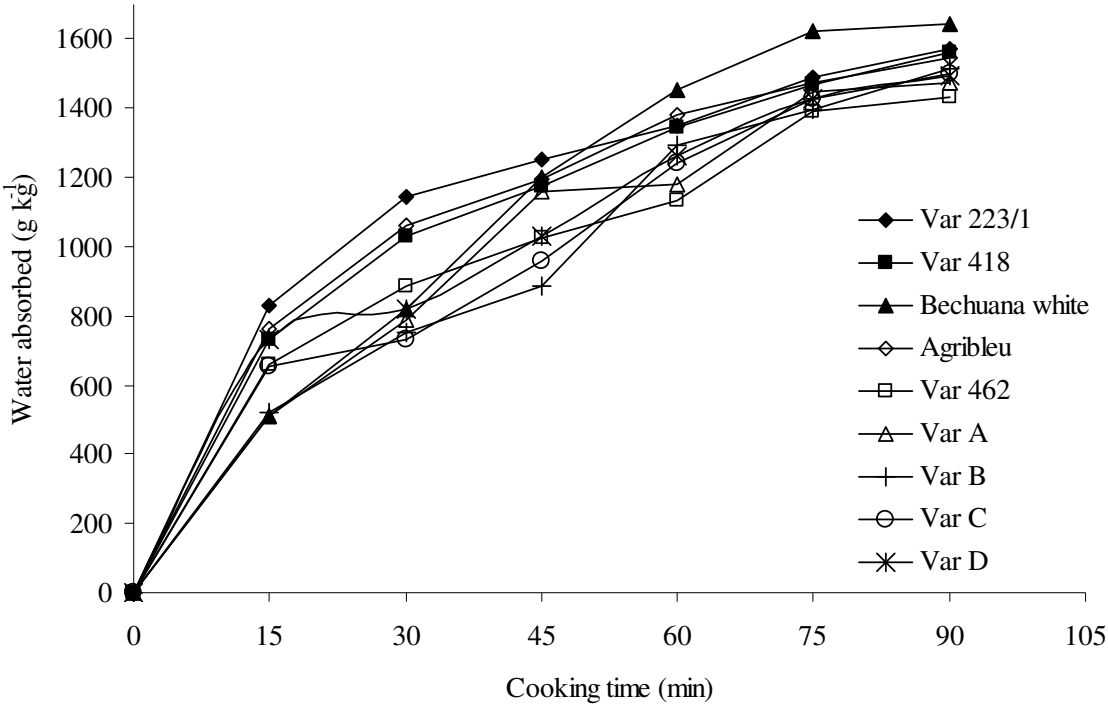

Figure 3.1.2 Water absorption pattern for 9 varieties of cowpeas during 90 min of cooking

Cowpeas contain approximately 48 % starch (Kerr *et al*., 2001), which undergoes the process of gelatinisation during cooking. During the first 15 min of cooking,

Bechuana white, Var. 462, Var. A, Var. B, and Var. C, absorbed significantly less amount of water as compared to Var. 223/1, Agribleu, Var.418 and Var. D cowpeas. There was no significant increase in the amount of water absorbed by all the cowpeas after 75 min of cooking, possibly due to the full hydration of the gelatinised starch. There were no significant differences in the amount of water absorbed by the cowpeas at 90 min of cooking.

3.1.3.3 Splitting of cowpea seeds during cooking

Overall, Bechuana white had significantly higher number of splits as compared to the rest of the varieties (Figure 3.1.3).

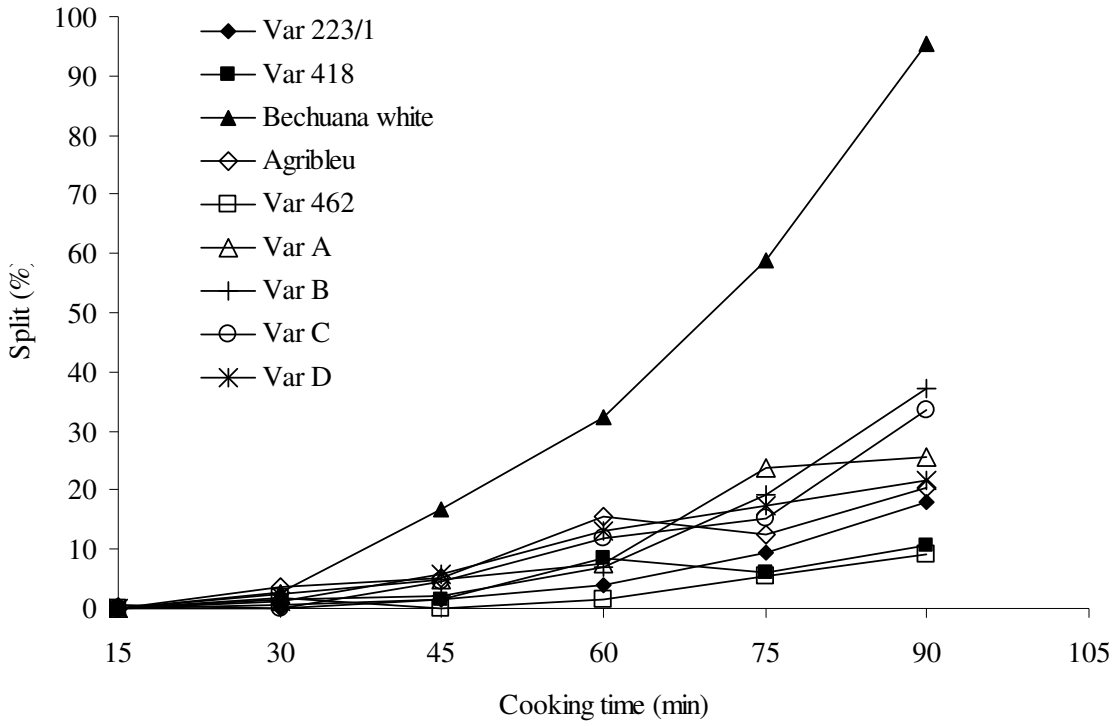

Figure 3.1.3 Splitting of cowpea seeds during 90 min of cooking

Extensive splitting of cooked whole cowpeas is an undesirable characteristic (Afoakwa *et al*., 2006). Splitting during cooking of cowpeas has been positively related to drained weight of cooked seeds (Taiwo *et al*., 1998). Cowpeas that have high water absorption properties during cooking tend to have a higher incidence of

splits. Splitting during cooking is an important quality characteristic for cowpeas that would be exposed to micronisation process.

3.1.3.4 Texture of cooked cowpea seeds

Texture is an important quality characteristic for cooked cowpeas (Sefa-Dedeh *et al*., 1978), and is usually used to measure the cooking time of cowpeas. Texture has been defined as the sensory and functional manifestation of structural, mechanical and surface properties of foods that is detected through the senses of vision, hearing, touch and kinesthetics (Szczesniak, 2002). Dry cowpeas are boiled until they attain a texture that is palatable for consumption. Table 3.1.1 shows that Var. 462 had a significantly harder texture as compared to the other cowpea varieties after 75 min of cooking. This may mean that Var. 462 may require longer cooking time to attain a softer texture that is similar to the other cowpea varieties. Since Var. 462 cowpeas did not have a hard shell, as indicated by the rapid hydration properties, the longer cooking time for Var. 462 could possibly be due to variations in compositional factors such as phytic acid, calcium and pectic substances.

3.1.4 Conclusions

Significant variation exists in the pattern of water uptake during soaking, cooked texture and splitting of the cooked cowpea varieties. These characteristics are most divergent between Bechuana white and Var. 462 cowpeas. Therefore these two varieties were selected for use in the subsequent study on the effect(s) of hydrothermal treatment (tempering and micronisation), on the structure of cowpeas and the physicochemical properties of carbohydrates and proteins as they relate to cooking quality characteristics.

3.2 Hydrothermal treatments of two cowpea (*Vigna unguiculata* L. Walp) varieties: effect of micronisation on physicochemical and structural characteristics[1]

Abstract

The effects of a hydrothermal treatment consisting of tempering (to 41 % moisture) and infrared heating to 153 °C (micronisation) on the structural and physicochemical characteristics of two cowpea varieties were studied. The untreated varieties had similar cooking times, although cooked Bechuana white was significantly ($P \leq 0.05$) softer and had a higher incidence of splitting than Var. 462 cowpeas. This may be due in part to differences in cotyledon structure affecting water uptake during cooking. The hydrothermal treatment changed the physical structure and chemical properties of the cowpea seeds. This led to significant ($P \leq 0.05$) reductions in cooking time of micronised (41 % moisture, 153 °C) Bechuana white and Var. 462 cowpeas, by 44 and 36 %, respectively, as compared with control samples. Micronisation (41 % moisture, 153 °C) caused physical fissuring of the seed coat and cotyledon and significantly ($P \leq 0.05$) reduced the bulk density of treated seeds. These changes in the physical structure significantly ($P \leq 0.05$) improved the initial water uptake during soaking and cooking, increased the enzyme-susceptible starch and reduced the protein solubility and hydration capacity of the cowpea seeds. Cooked (60 min) micronised (41 % moisture, 153 °C) cowpeas also had significantly ($P \leq 0.05$) more splits and a significantly ($P \leq 0.05$) softer texture than control samples.

Key words: cowpea seeds, micronisation, cellular structure, cooking time, texture, splits, water absorption

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3.2.1 Introduction

In sub-Saharan Africa the problem of protein-energy malnutrition still exists despite the availability of legumes, such as cowpeas which are a substantial source of affordable protein, B vitamins and minerals (Phillips *et al*., 2003). The utilisation of dried cowpeas is limited in part owing to long cooking times (up to 160 min, CT_{50}[2]), which translates into increased demands on energy and time required for meal preparation (Akinyele *et al*., 1986). These problems are prevalent in southern Africa where dry cowpeas are usually boiled without soaking (Demooy & Demooy, 1990). The cooking time of legumes has been related in general to starch gelatinisation, protein denaturation and degradation of the middle lamella between parenchyma cells of the cotyledon (Sefa-Dedeh & Stanley, 1979a). Gelatinisation of cowpea starch in the cotyledon during cooking is considered to be one of the major physicochemical and structural changes that must occur prior to softening of cowpeas (Liu *et al*., 1993a). Heat-induced denaturation and coagulation of proteins have to occur after starch gelatinisation otherwise it may result in a physical barrier that could restrict water uptake and swelling of the starch granules (Liu *et al*., 1993a). Another major physicochemical and structural change that must occur prior to softening of cowpeas is the disintegration of the middle lamella during cooking of cowpeas. This is evidenced by the separation of cells along the cell wall observed using scanning electron microscopy (Sefa-Dedeh *et al*., 1978; Sefa-Dedeh & Stanley, 1979a).

Cooking of dry cowpeas is a process that involves both rehydration and heating. These two processes may take place simultaneously as in the case of cooking cowpeas without pre-soaking, or rehydration may be done prior to heating through soaking. Therefore, in order to reduce the cooking time of legumes, methods have been proposed that are based on facilitating the gelatinisation of starch by supplying water to the starch granules early in the cooking process, e.g. by soaking in water at 25 °C for different time intervals (Sefa-Dedeh *et al*., 1978; Jackson & Varriano-Marston, 1981) or at elevated temperatures between 45 and 75 °C for 1 h (Taiwo *et al*., 1997b). However, Demooy and Demooy (1990) reported that 12 h of soaking at 15 °C did not significantly reduce the cooking time of cowpeas. Furthermore, legumes such as cowpeas have also been soaked in dilute organic acid or sodium salt solutions in order

[2] CT_{50} . Cooking time determined using a Mattson Bean Cooker using the 50 % cooked stage

to displace calcium ions (Onigbinde & Ojeabulu, 1999). These solutions reduce the formation of calcium pectates in the middle lamella during cooking (Rockland & Jones, 1974). The formation of calcium pectates would limit the desirable cell separation and increase the time required to attain a soft texture.

Another method that has potential of reducing cooking time of legumes is micronisation. Micronisation is an infrared (IR) heat treatment, which when used on moisture-conditioned grains and legumes, has been reported to reduce cooking times by 50 % for Laird lentils (Cenkowski & Sosulski, 1997; Arntfield *et al*., 2001) and 30 % for split peas (Cenkowski & Sosulski, 1998). Reports show that micronisation of preconditioned seeds pregelatinises starch and denature proteins in treated split peas (Cenkowski & Sosulski, 1998) and lentils (Arntfield *et al*., 2001). Physically, micronisation has been shown to increase the rate of rehydration during soaking of IR-treated Pinto beans (*Phaseolus vulgaris*) at 22 °C (Abdul-Kadir *et al*., 1990) and cooking of micronised split peas (Cenkowski & Sosulski, 1998). Although micronisation (17 % moisture, 99 and 107 °C) improved the hydration rate of IR-heated Pinto beans, it also increased the cooking time (Abdul-Kadir *et al*., 1990). Increased cooking time, a hardening of seed texture, and reduced water absorption capacity have also been reported in micronised (17 % moisture, 69, 88 and 90 °C) chickpeas (Sarantinos & Black, 1996). Preliminary trials involving micronisation of moisture-conditioned cowpeas indicated a strong effect of genotype and that the hydrothermal process effectively shortened cooking time. There is a possibility that cowpeas could be processed into a quicker cooking legume, thus reducing energy and time requirements and leading to increased utilisation and consumption.

In order to understand the mechanisms whereby micronisation of moisture-conditioned seeds induces changes in cooking characteristics of legumes, this study examined the effect of micronisation on the structural and physicochemical characteristics of moisture-conditioned cowpeas and the resulting changes in cooking characteristics of two cowpea varieties with different physicochemical characteristics.

3.2.2 Materials and methods

3.2.2.1 Raw materials

During a preliminary study as reported in section 3.1, nine cowpea varieties (from South Africa and Malawi) were screened in terms of their chemical composition, textural properties during cooking, and water absorption during soaking and cooking. Two varieties differing in these physicochemical characteristics were chosen for the study. Cowpea varieties with smooth seed coats; Var. 462 (maroon colour) and Bechuana white (cream colour) were obtained (within 1 month of harvest) from Bunda College of Agriculture in Malawi and Agricol in Potchefstroom, South Africa, respectively. Cowpea samples were cleaned to remove chaff and shrivelled and broken seeds. The cleaned seeds were packed in polypropylene bags and stored at 4 °C until the time of use.

3.2.2.2 The hydrothermal process

The micronisation parameters (moisture content and microniser settings) were selected in order to obtain relatively stable products without burning (Phadi, 2004) as illustrated in Figure 3.2.1. Cowpeas were tempered to approximately 41 % moisture content by steeping in deionised water (1:5 w/v) at 22 °C for 2 h for Var. 462 and 6 h for Bechuana white. The cowpeas were removed from the water, blotted dry on absorbent paper and held for a further 12 h at 22 °C to equilibrate the moisture throughout the seed. The cowpeas were micronised in 160 g batches using a tabletop microniser with three 2 kW Phillips IR lamps (Technilamp Pty, Johannesburg, South Africa) operating at 66.7 % output. The microniser was preheated for 20 min before micronising the cowpeas in a single layer (21 cm from energy source) for 6 min to a final surface temperature of 153 °C. Temperature during micronisation was monitored using thermocouples attached to a Grant Squirrel 800 data logging system (Monitoring and Control Lab, Johannesburg, South Africa). After micronisation the cowpeas were spread on a tabletop and cooled to room temperature for 1 h before being packed in zipper bags (Plastilon Packaging, Pretoria, South Africa) and kept at 22 °C. All samples were analysed within 2 days.

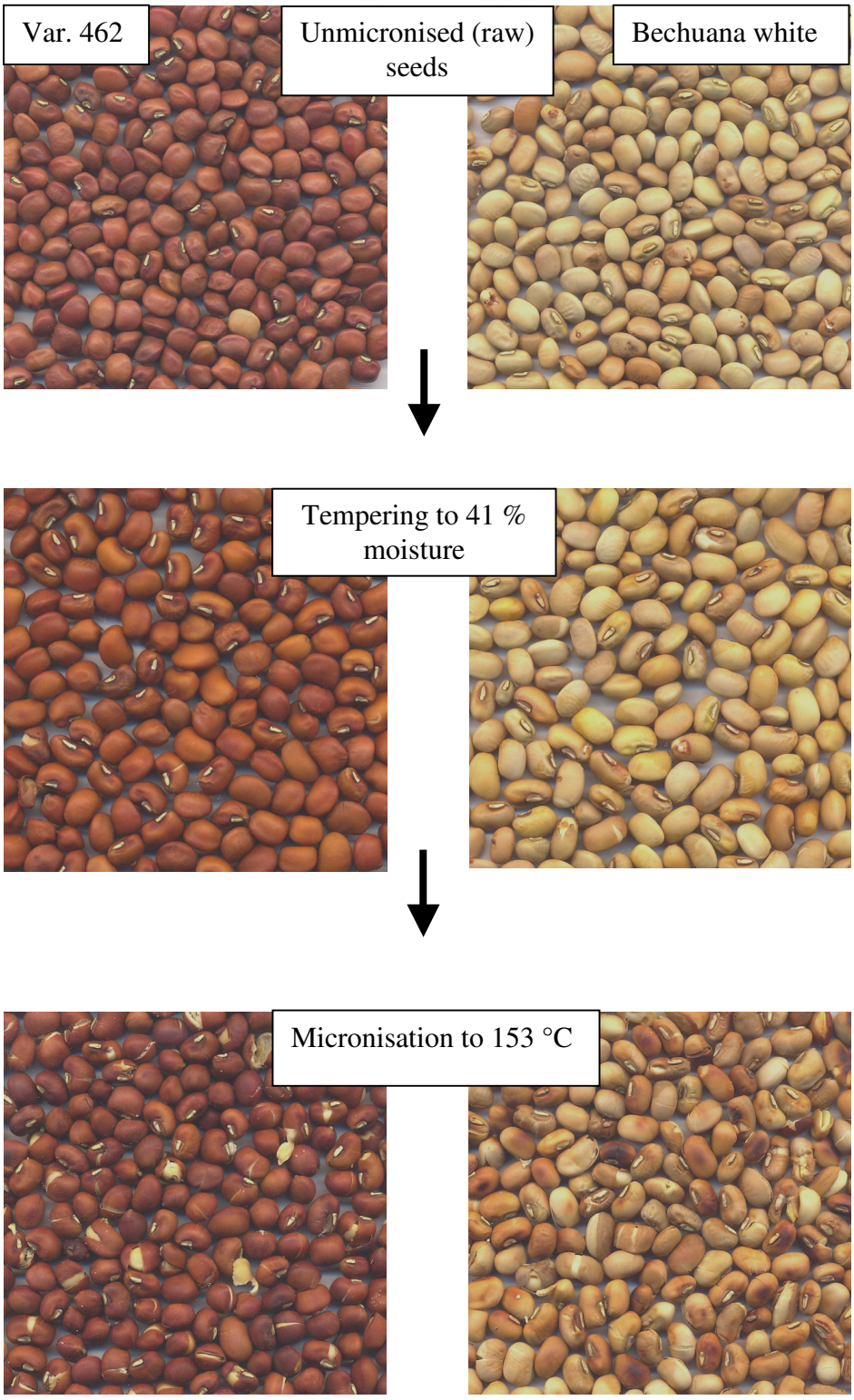

Figure 3.2.1 Flow diagram for the hydrothermal process used in micronising (41 % moisture, 153 °C) cowpea samples

3.2.2.3 Determination of seed moisture content

The moisture content in raw, tempered and micronised (41 % moisture, 153 °C) cowpea seeds was determined according to the method of Ajibola *et al*. (2003) as reported in section 3.1.2.1.

3.2.2.4 Determination of crude protein content

Crude protein content in the cowpeas was determined using the Dumas method as reported in section 3.1.2.2.

3.2.2.5 Determination of protein solubility

Reduction in protein solubility has been associated with denaturation, therefore protein solubility of the samples was determined according to the method of Arntfield *et al*. (1997) with minor modifications. A sample dispersion of 5 % (w/v) in 0.5 M NaCl at pH 7 was centrifuged at 3500 *x g* for 10 min. The nitrogen content in the supernatant was determined using a Leco FP 528 nitrogen analyser (Leco Africa Pty, Kempton Park, South Africa).

3.2.2.6 Determination of total and enzyme-susceptible starch

The total starch content in unmicronised and micronised (41 % moisture, 153 °C) cowpeas was determined according to the method of McCleary, Gibson and Mugford (1997) using the Megazyme total starch assay kit (Megazyme International, Wicklow, Ireland). The method was slightly modified to measure the percentage of enzyme susceptible starch in the uncooked micronised (41 % moisture, 153 °C) and unmicronised cowpeas, which is a measure of starch gelatinisation (Hoover & Manuel, 1996b). Enzyme-susceptible starch was determined by digesting the samples with thermo stable α-amylase and incubating at 37 °C.

3.2.2.7 Determination of seed bulk density

Cowpea seed bulk density was determined according to the method described by Fasina *et al*. (1999). The cowpea seeds were placed in a metal funnel and allowed to flow from 15.5 cm height into a 500 ml metal cup. The grains were levelled without pressing with a metal scraper. The ratio of the weight of sample in the metal cup to the volume of the cup was expressed as kg m^{-3} and recorded as bulk density.

3.2.2.8 Determination of water absorption during soaking and hydration capacity

Water absorption during soaking was determined according to a modified method of Agbo *et al*. (1987) as reported in section 3.1.2.4. The amount of water (g) absorbed by 10 g of cowpeas after 18 h of soaking reflected the hydration capacity (g water kg^{-1} cowpeas) as reported by Wang, Daun and Malcolmson (2003).

3.2.2.9 Determination of water absorption during cooking

The amount of water absorbed during cooking was determined according to a modified method of Cenkowski and Sosulski (1997) as reported in section 3.1.2.5. The boiled cowpea samples were then used to determine splitting and texture.

3.2.2.10 Determination of splitting during cooking

The tendency of seeds to split during cooking was determined according to the method of van Buren *et al*. (1986) as reported in section 3.1.2.6.

3.2.2.11 Determination of seed texture during cooking

Texture was measured as the work (area under the curve, N mm) done to cut individual cowpea seeds through a distance of 5 mm at a speed of 4 mm s^{-1} based on the method of Sefa-Dedeh *et al*. (1978). A TA-XT2 texture analyser (Stable Micro Systems, Godalming, UK) with A/CKB craft knife adapter mounted with Hilite® heavy-duty blades (Hilite Hardware, Pretoria, South Africa) was used to measure texture. A single cowpea seed was positioned on its side and cut below the hilium along the cross-section. The blade was replaced after 5 determinations. For each sample of unmicronised and micronised (41 % moisture, 153 °C) cowpeas cooked for 15, 30, 45, 60, 75 and 90 min, 10 seeds were measured.

3.2.2.12 Determination of cooking time

A custom made Mattson bean cooker (Pretoria, South Africa) as described by Wang *et al*. (2003) was used to determine the cooking time of micronised (41 % moisture, 153 °C) and unmicronised cowpea samples. For each test sample, 25 cowpea seeds were positioned in the perforations of the cooker and placed in an aluminium pan with 1500 ml of deionised water and cooked. The cooking time of the cowpeas was recorded as the moment when 80 % of the pins had fallen through the softened seeds.

3.2.2.13 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of cowpea samples (unmicronised, micronised (41 % moisture, 153 °C), unmicronised samples cooked for 30 and 60 min, micronised (41 % moisture, 153 °C) samples cooked for 15 and 30 min) was conducted according to the method of Sefa-Dedeh and Stanley (1979a) with modifications. These cooking times represent half-cooked and fully cooked stages of unmicronised and micronised (41 % moisture, 153 °C) samples, respectively. Cowpea samples (3 seeds) were fixed in 2.5 % glutaraldehyde in 0.075 mol L^{-1} phosphate buffer, pH 7.4, for 24 h at 4 °C. The samples were then rinsed three times using the same buffer at 15 min intervals. Dehydration of the samples was accomplished by 20 min sequential changes in 50, 70, 90 % ethanol followed by three 20 min changes in 100 % ethanol. The samples were dried using the critical point drying technique and mounted on aluminium stubs with the aid of double-sided carbon tape, followed by coating with gold. The coated samples were viewed and photographed using a JSM-840 scanning electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 5.0 kV.

3.2.2.14 Enzyme digestion and environmental scanning electron microscopy (ESEM)

In order to elucidate the presence of protein and pectic substances outside the parenchyma cells, the control cowpea seeds were cooked for 30 min and digested with pectinase or protease. The cooked cowpea seeds were cut in half across the hilum. A sample was digested for 1 h in 2 ml of pectinase (Pectinase from Rhizopus Sp, CAS No. 9032-75-1, Sigma Inc, St Louis Missouri) at room temperature while another sample was digested in 10 ml of protease (1g ml^{-1}, Sigma EC 232-752-2, in pH 7.5 phosphate buffer) for 1 h at 37 °C. The cooked seeds were mounted onto 13 mm diameter aluminium stubs using double adhesive tape. Samples were viewed and photographed using an environmental scanning electron microscope (ESEM, Electroscan model E-3, Wilmington, MA) at an accelerating voltage of 15.0 kV following the method reported by McDonough and Rooney, 1999.

3.2.2.15 Statistical analysis

The experimental work was conducted on duplicate samples and repeated three times to generate data that were analysed using Statistica version 6.0 (StatSoft Inc, Tulsa,

USA). Analysis of variance (ANOVA) of the (2 x 2)-factor experiment was used to evaluate the data based on a 5 % level of significance. When the F-test was significant, differences between means were determined using the least significant difference (LSD) test. Two-dimensional regression was used to determine correlations between texture, splitting and water absorbed at 15 min intervals, from 15 min to 90 min of cooking. The regression was tested for significance at 5 %.

3.2.3 Results and discussion

3.2.3.1 Soaking and hydration characteristics

Var. 462 cowpeas had a significantly ($P \leq 0.01$) higher uptake of water than Bechuana white cowpeas (Figure 3.2.2) during 6 h of soaking. After 6 h of soaking, unmicronised Var. 462 cowpeas absorbed 1130 g water kg^{-1}, which was significantly ($P \leq 0.05$) higher than 532 g water kg^{-1} absorbed by Bechuana white cowpeas.

The hydration capacity of Var. 462 cowpeas was also significantly ($P \leq 0.05$) higher than that of Bechuana white cowpeas (Table 3.2.1). Water imbibition during soaking of legumes such as cowpeas and beans is a process that is related to physical characteristics of the seed and its component macromolecules, specifically proteins (Sefa-Dedeh & Stanley, 1979b). Var. 462 cowpeas had significantly ($P \leq 0.05$) higher crude protein content than Bechuana white cowpeas, although the percentage of soluble protein was similar (Table 3.2.1). Protein is one of the major macromolecules in cowpeas. Proteins are considered to play a major role in water absorption during soaking (Sefa Dedeh & Stanley, 1979b) hence Var. 462 showed higher water absorption than Bechuana white cowpeas (Figure 3.2.2).

In addition to variations in protein content, Var. 462 cowpeas had a significantly lower bulk density than Bechuana white cowpeas (Table 3.2.1). The lower bulk density implied that there were more air spaces in Var. 462 than in Bechuana white cowpeas. The SEM micrographs of the cotyledon structure of unmicronised Var. 462 cowpeas (Figure 3.2.3 (a)) showed loosely packed cells with larger intercellular spaces as compared to the compact cotyledon structure of Bechuana white cowpeas (Figure 3.2.3 (b)).

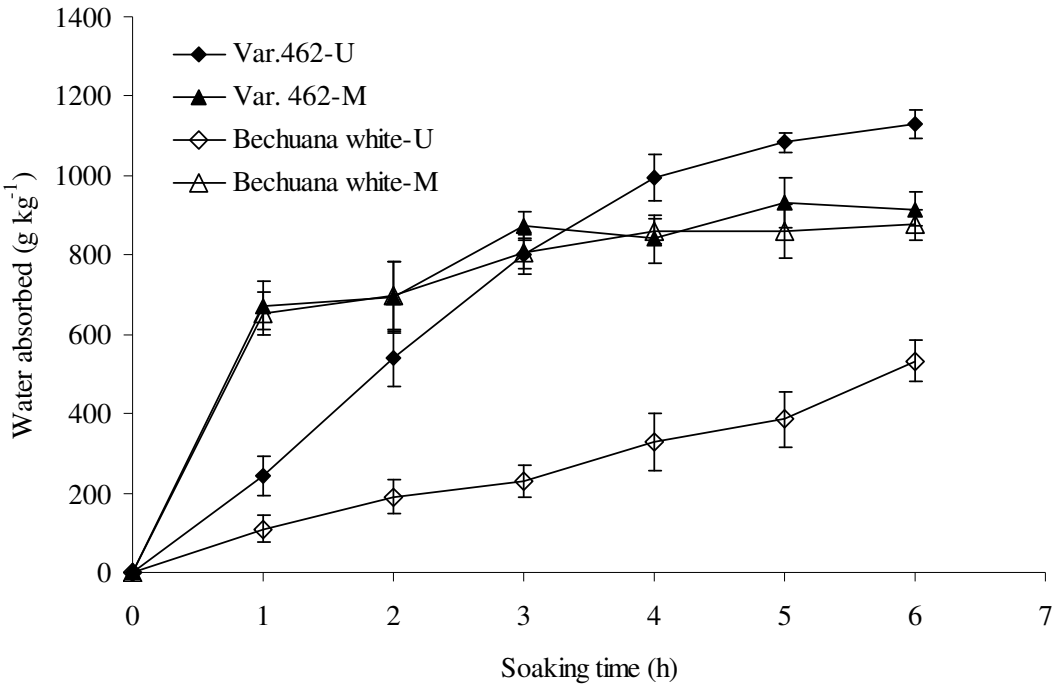

Figure 3.2.2 Effect of micronisation (41 % moisture, 153 °C) on water absorption during the soaking of Var.462 and Bechuana white cowpeas at 22 °C (vertical bars indicate standard deviations of the means, and U = Unmicronised (raw), M = Micronised (41 % moisture, 153 °C))

This difference in cotyledon structure could be responsible for the difference in water uptake patterns of the two cowpea samples when soaked at 22 °C (Sefa Dedeh and Stanley, 1979b). The initial rapid water uptake during soaking at room temperature has been reported to be due to the filling up of free capillary and intercellular spaces (Phlak *et al.*, 1989), hence Var. 462 with more capillary and intercellular spaces showed a higher water uptake during the 6 h of soaking than Bechuana white cowpeas.

Table 3.2.1 Effect of variety and micronisation (41 % moisture, 153 °C) on physicochemical properties of cowpea seeds

Physicochemical characteristics	Var. 462		Bechuana white	
	[y]Unmicronised	[z]Micronised	[y]Unmicronised	[z]Micronised
Moisture ($g\ kg^{-1}$)	117^{b} (0.8)	157^{a} (25.6)	89^{c} (3.8)	123^{b} (21.5)
*Crude protein ($g\ kg^{-1}$ db)	257^{b} (2.4)	277^{a} (8.6)	240^{c} (1.8)	262^{b} (6.5)
Protein solubility (%)	85^{a} (8.9)	33^{b} (6.5)	88^{a} (7.0)	34^{b} (10)
*Starch ($g\ kg^{-1}$ db)	439^{b} (17)	489^{a} (30)	445^{b} (23)	459^{b} (22)
ESS (%)	14^{d} (1)	86^{a} (3.1)	21^{c} (6.8)	68^{b} (2.8)
Bulk density ($kg\ m^{-3}$ db)	801^{b} (2.6)	638^{d} (11.7)	870^{a} (4.4)	658^{c} (9.2)
Cooking time (min)	59^{a} (0.2)	38^{b} (2.4)	57^{a} (1.9)	32^{c} (6.9)
Hydration capacity ($g\ kg^{-1}$ cowpeas db)	1341^{a} (30)	975^{c} (26)	1179^{b} (55)	928^{c} (50)

ESS= Enzyme-susceptible starch; [y]Unmicronised (raw), [z]Micronised (41 % moisture, 153 °C); means followed by the same letter within a row are not significantly different at level $P \leq 0.05$; standard deviation of the means are in parenthesis; * = The increase in these values does not reflect the effect of micronisation

3.2.3.2 Cooking characteristics

Cooking time of the unmicronised cowpeas was not significantly different between the two varieties, as determined by the Mattson bean cooker (Table 3.2.1). These cooking time values are within the ranges (36 to 56 min) reported by Demooy and Demooy (1990) for 7 unsoaked cowpea varieties. Cooking time refers to the time required for the cowpeas to attain a level of softness, a textural parameter.

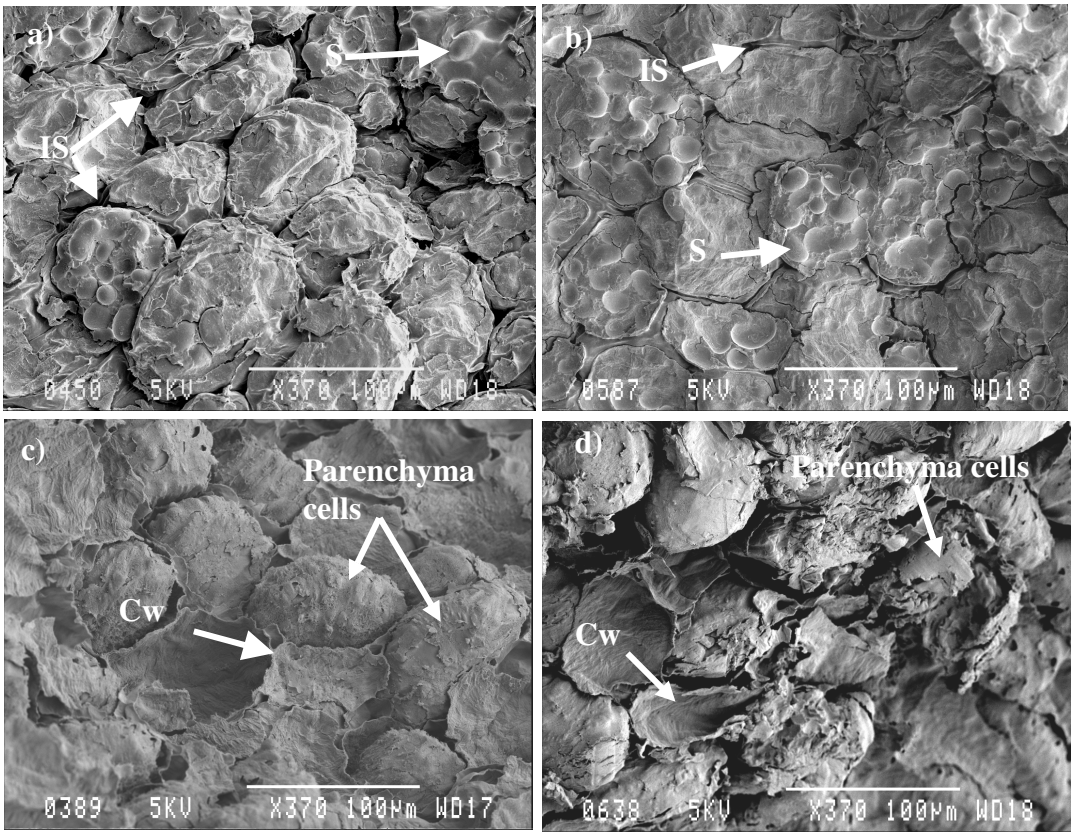

Figure 3.2.3 Varietal differences and micronisation (41 % moisture, 153 °C) effect on the cotyledon structure of two cowpea varieties: cotyledon cross-section of raw Var. 462 (a); raw Bechuana white (b); micronised Var. 462 (c); micronised Bechuana white (d); Cw = Cell wall, IS = Inter- cellular space, S = Starch granule

The texture (work required to cut through a seed) of the two cowpeas varieties became increasingly softer with increasing cooking time (Figure 3.2.4). This is not surprising since there was a significant ($P \leq 0.0001$) negative correlation between cooking time and texture (Var. 462 cowpeas, $r = -0.90$; Bechuana white cowpeas, $r = -0.88$). The decrease in work required to cut through the seed may be related to the separation of cotyledon parenchyma cells.

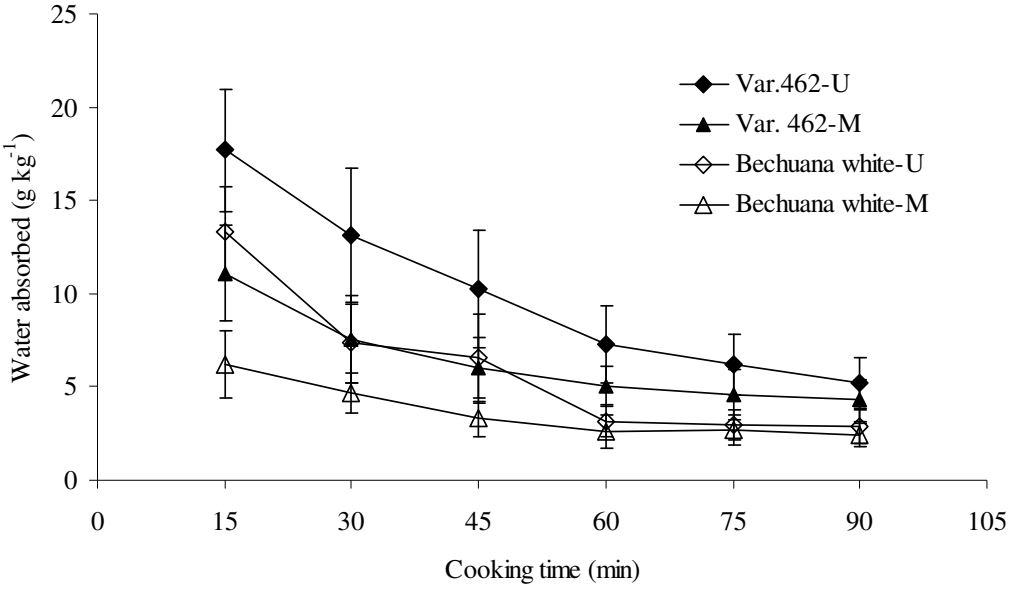

Figure 3.2.4 Effect of micronisation (41 % moisture, 153 °C) on the texture (work in Nmm) of Var. 462 and Bechuana white cowpeas during 90 min of cooking (vertical bars indicate standard deviations of the means and U= Unmicronised (raw) and M = Micronised (41 % moisture, 153 °C))

The disintegration of the middle lamella of the cells that were cemented together is shown in Figures 3.2.5(a), 3.2.5(b), 3.2.5(e) and 3.2.5(f). Earlier researchers have also observed that the disintegration of the middle lamella corresponds to the softening of cowpeas during cooking (Sefa Dedeh & Stanley 1979a). The disintegration of the middle lamella in cowpea cotyledon cells probably allows the cells to separate readily during mechanical fracturing, resulting in a softer texture.

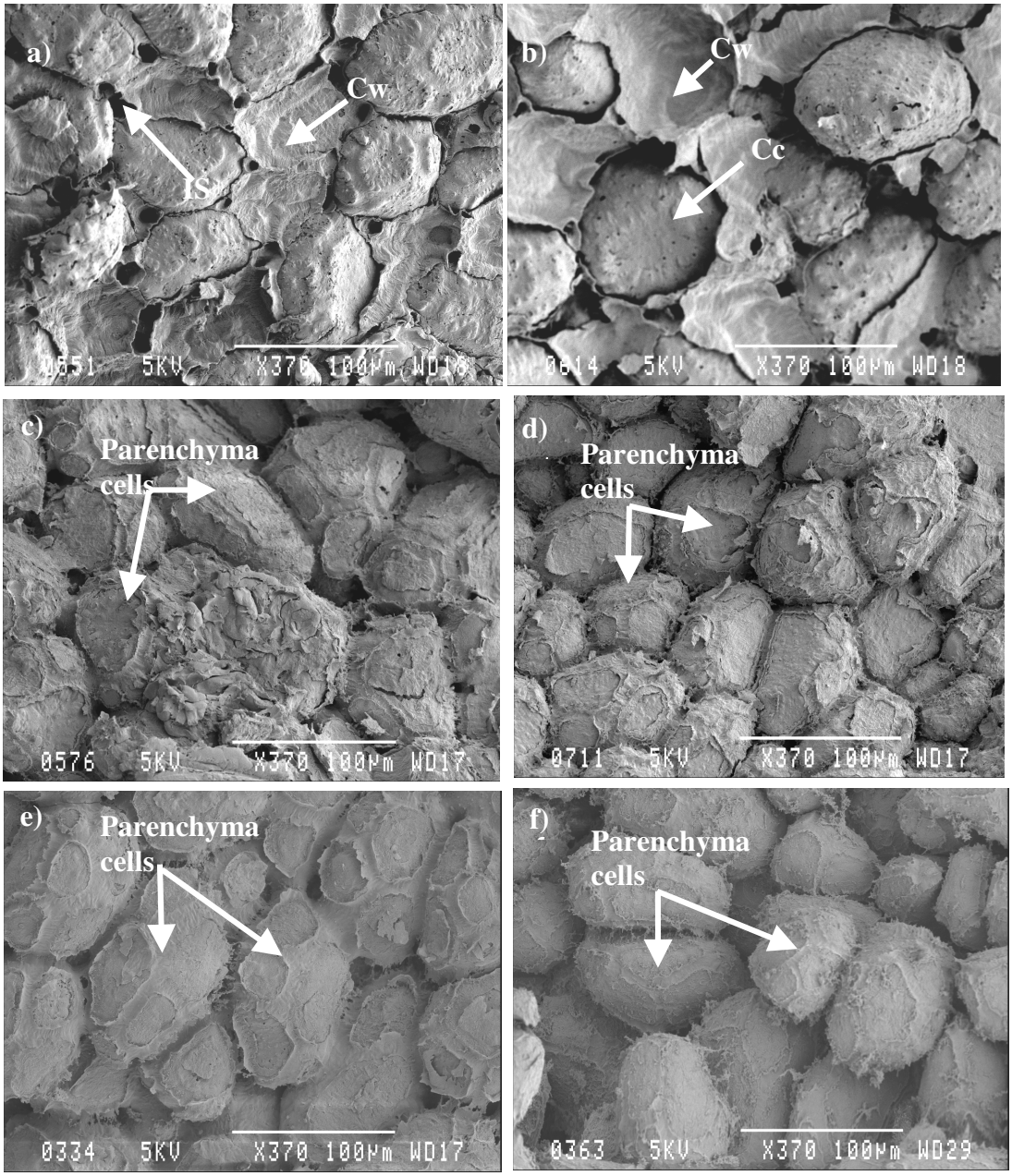

Figure 3.2.5 **Cotyledon cross sections of unmicronised (raw) and micronised (41 % moisture, 153 °C) cowpeas at half cooked and fully cooked stages: 30 min cooked unmicronised (a) Var. 462 and (b) Bechuana white; 15 min cooked micronised (c) Var. 462 and (d) Bechuana white; 60 min cooked micronised (e) Var. 462 and (f) Bechuana white; 30 min cooked micronised (g) Var. 462 and (h) Bechuana white; Cw = Cell wall; Cc = Cellular contents; and IS = intercellular spaces**

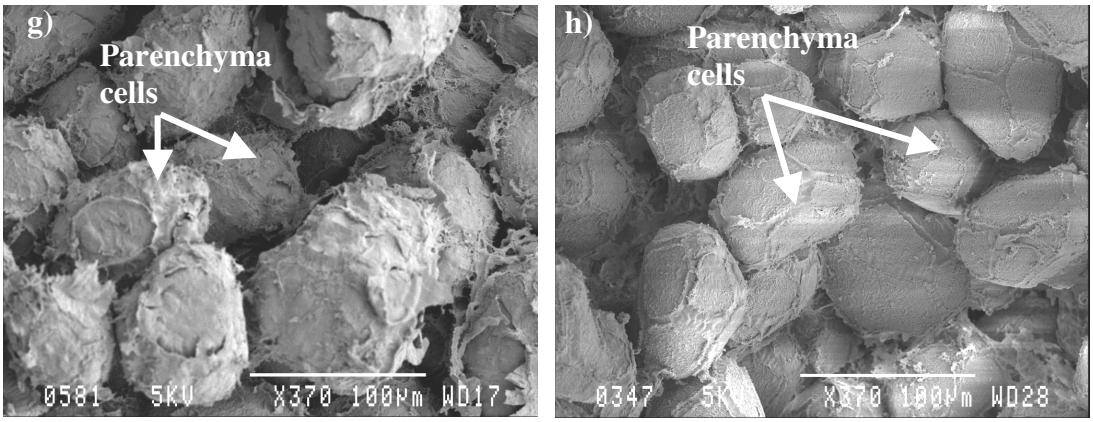

Figure 3.2.5 Continued

In addition to the solubilisation of the middle lamella during cooking, it appears that protein gelation outside the cell also provides some form of adhesive between the cells after cooking. Through the use of ESEM in combination with enzyme digestion it was evident that protein material was present outside the cells (Figure 3.2.6 (a), 3.2.6 (b) & 3.2.6 (c)) and was digested by proteinase.

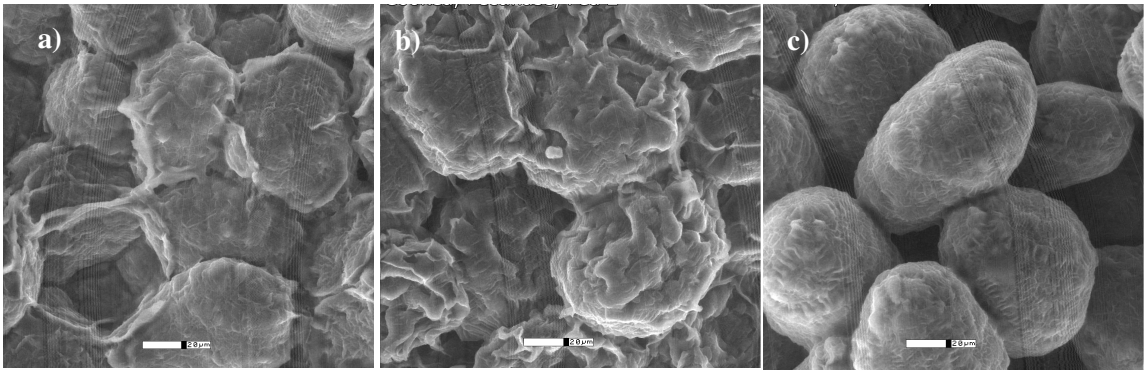

Figure 3.2.6 Environmental scanning micrographs (ESEM) of unmicronised (raw) cowpea seed cotyledon cooked for 30 min showing the effect of enzyme digestion: (a) no enzyme treatment, (b) pectinase, (c) proteinase; bar =20 µm

Unmicronised Bechuana white cowpeas had a significantly softer texture than unmicronised Var. 462 cowpeas after 60 min of cooking, which is close to the cooking times determined using the Mattson bean cooker which were 59 and 57 min, respectively (Tables 3.2.1 & 3.2.2). Differences in the cooked texture of cowpeas were also reported by Demooy and Demooy (1990), where some cowpea varieties had a smooth mushy texture with a softened seed coat when cooked. This was observed with Bechuana white cowpeas. Other cowpeas are reported to have a tough seed coat even after the cotyledon has become soft and mushy (Demooy & Demooy, 1990). This pattern was observed with cooked Var. 462 cowpeas.

Table 3.2.2 Effect of variety and micronisation (41 % moisture, 153 °C) on cooking characteristics of cowpeas after 60 min of cooking

Variety	Micronisation	Water uptake (g H_2O kg^{-1} seed)	Texture (N mm)	Splits (%)
Var. 462	[y]Unmicronised	1140^{c} (62)	7.3^{a} (2.0)	2.2^{d} (1.2)
	[z]Micronised	1248^{b} (92)	5.0^{bc} (1.1)	34.4^{c} (4.9)
Bechuana	[y]Unmicronised	1390^{a} (22	3.2^{c} (0.9)	45.3^{b} (9.2)
white	[z]Micronised	1292^{b} (89)	2.6^{d} (0.9)	61.9^{a} (7.6)

[y]Unmicronised = raw; [z] = Micronised (41 % moisture, 153 °C); means followed by the same letter within a column are not significantly different at level $P \leq 0.05$; standard deviations of the means are in parenthesis

The difference in the texture of cooked unmicronised Var. 462 and Bechuana white cowpeas could also possibly be due to the significantly higher amounts of water absorbed during cooking by Bechuana white cowpeas (Figure 3.2.7). During the initial 15 min of cooking, there was no difference in amount of water absorbed between the two varieties. Thereafter, unmicronised Bechuana white cowpeas absorbed more water than unmicronised Var. 462 cowpeas. Availability of water in the cotyledon facilitates heat transfer and is necessary for starch gelatinisation during cooking (Arntfield *et al*., 1997).

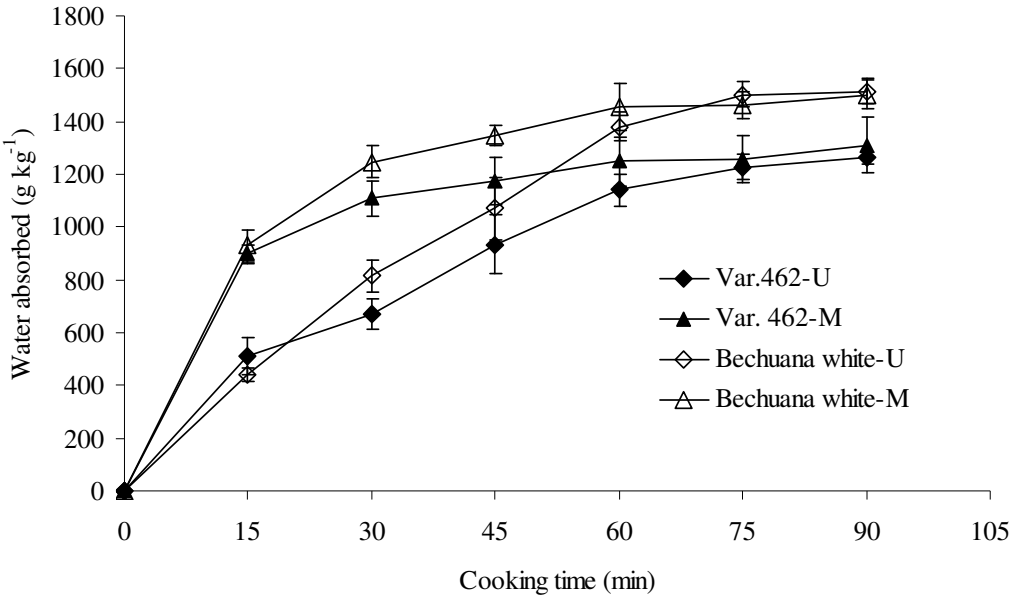

Figure 3.2.7 Effect of micronisation (41 % moisture, 153 °C) on water absorption during 90 min of cooking Var. 462 and Bechuana white cowpeas (vertical bars indicate standard deviations of the means, U = Unmicronised (raw), M= Micronised (41 % moisture, 153 °C))

Although unmicronised Var. 462 cowpeas absorbed more water during soaking at 22 °C (Figure 3.2.2), Bechuana white cowpeas absorbed more water during cooking (Figure 3.2.7). Bechuana white cowpeas had higher ($P \leq 0.05$) enzyme-susceptible starch content than Var. 462 cowpeas (Table 3.2.1), and the greater amount of damaged starch may have contributed to increased water absorption.

High water absorption by cowpeas during cooking has also been associated with increased incidence of splits (Taiwo *et al*., 1998). After 15 min of cooking, unmicronised Bechuana white cowpeas had significantly ($P \leq 0.05$) more splits than Var. 462 cowpeas (Figure 3.2.8). The percentage of split cowpeas for both varieties correlated ($r= 0.73$; $P \leq 0.05$) with water absorption during cooking.

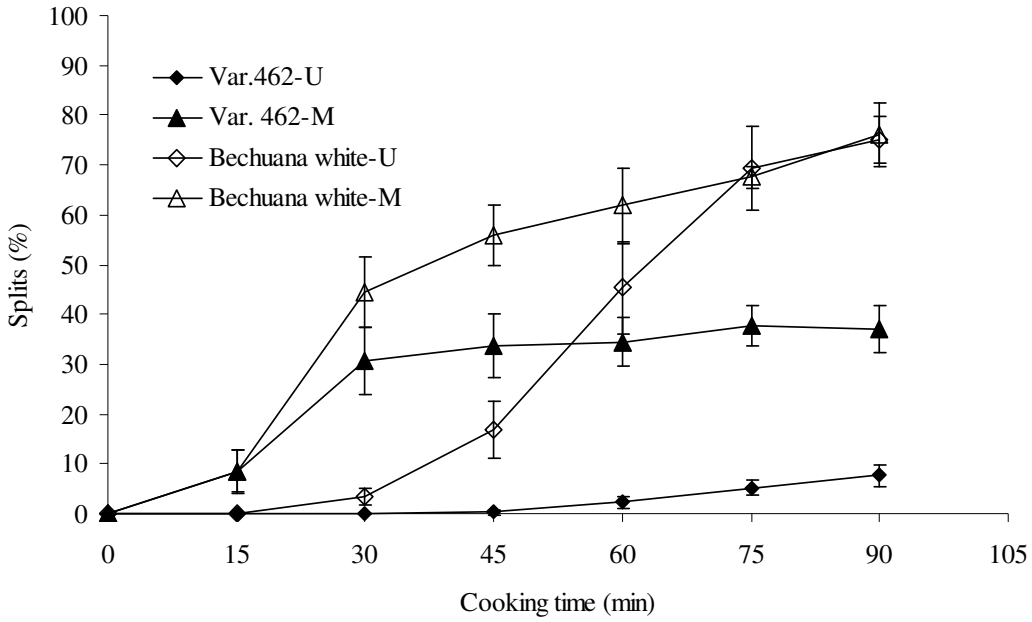

Figure 3.2.8 Effect of micronisation (41 % moisture, 153 °C) on splitting of Var. 462 and Bechuana white cowpeas during 90 min of cooking (vertical bars indicate standard deviations of the means; U = Unmicronised (raw); M = Micronised (41 % moisture, 153 °C))

The pattern of splitting in unmicronised cowpeas seemed to start with cracking of the seed coat through to the cotyledon transversely below the hilum (Figure 3.2.9) or along the edges resulting in the separation of the two cotyledons.

Figure 3.2.9 Cooked Bechuana white (unmicronised) seeds showing the pattern of splitting during cooking

Taiwo *et al*. (1997b) reported a similar pattern of splitting in cowpeas. Moreover, Taiwo *et al*. (1998) reported that % splits in cowpeas increased with softening texture and increasing drained weight of cooked cowpeas. The % splits in unmicronised Bechuana white cowpeas was negatively correlated with texture ($r= -0.81$; $P \leq 0.001$) and positively correlated with water uptake during cooking ($r= 0.92$; $P \leq 0.001$). Water uptake during cooking results in increased volume as the cotyledon cells absorb more water and expand. The expanding cells require more space and the continuity of the testa and/or cotyledon is compromised or broken. Bechuana white cowpeas had higher bulk density initially and higher water uptake during cooking; therefore the dense seeds expanded and transversal splitting of cotyledons occurred during cooking (Table 3.2.2). Conversely, Var. 462 cowpeas were less dense, had lower water uptake during cooking and showed less disruption in the integrity of the cotyledon and testa during cooking (Table 3.2.2). Similarly, Heil, McCarthy and Özilgen (1992) observed that the splitting tendency in beans (*Phaseolus vulgaris*) increased with increasing seed density.

It was observed that the split Bechuana white cowpeas tended to form lumps during extended cooking, a phenomenon that was previously reported in cowpeas (Taiwo *et al*., 1997b). The lumps consisted of several split and non-split cowpeas. The functionality of the major components of cowpeas changes during cooking, i.e. starch is being gelatinised and proteins are being denatured becoming less extractable. It is postulated that starch dispersion and retrogradation could result in aggregation of the split and non split cowpeas.

3.2.3.3 Effect of micronisation (41 % moisture, 153 °C) on soaking and hydration characteristics of cowpeas

The hydrothermal treatment increased the amount of water absorbed at 22 °C during the first hour of soaking in both varieties (Figure 3.2.2). The distinctive rapid increase in water uptake during the first hour of soaking is due to fissures in the seed coat integuments (compare Figures 3.2.10(a), to 3.2.10(l)) and the significant ($P \leq 0.05$) reduction in bulk density of micronised (41 % moisture, 153 °C) seeds (Table 3.2.1). In unmicronised cowpeas the seed coat is the first barrier to water transfer into the cotyledon (Sefa-Dedeh & Stanley, 1979b; Fasina *et al*., 1999).

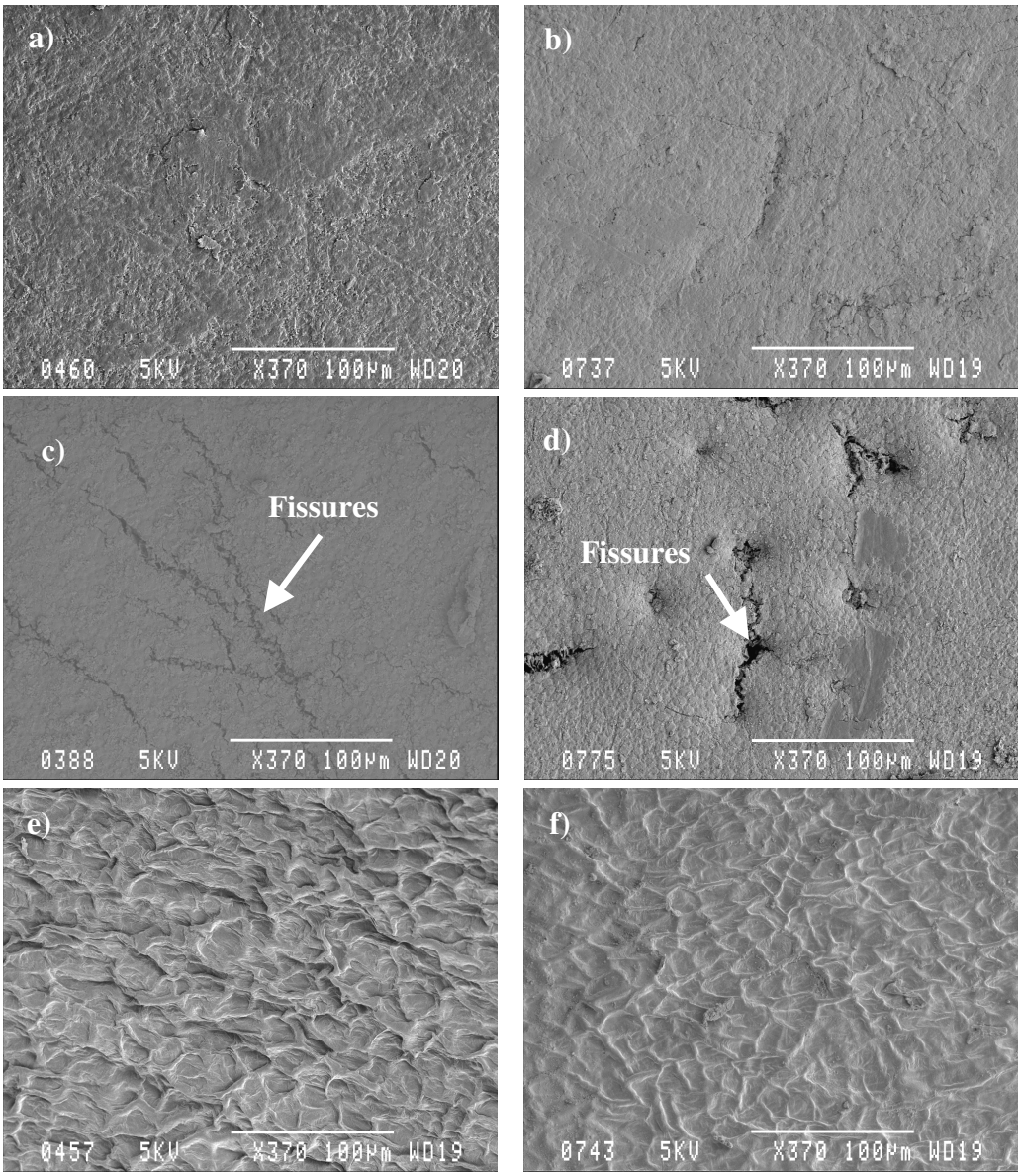

Figure 3.2.10 Effect of micronisation (41 % moisture, 153 °C) on structure of cowpea seed coat layers: outer surface of unmicronised (a) Var. 462 and (b) Bechuana white; outer surface of micronised (c) Var. 462 and (d) Bechuana white; inner surface of outer integument of unmicronised (e) Var. 462 and (f) Bechuana white, inner surface of outer integument of micronised (g) Var. 462 and (h) Bechuana white; outer surface of inner integument of unmicronised (i) Var. 462 and (j) Bechuana white; outer surface of the inner integument of micronised (k) Var. 462 and (l) Bechuana white; Unmicronised = raw; Micronised = 41 % moisture, 153 °C

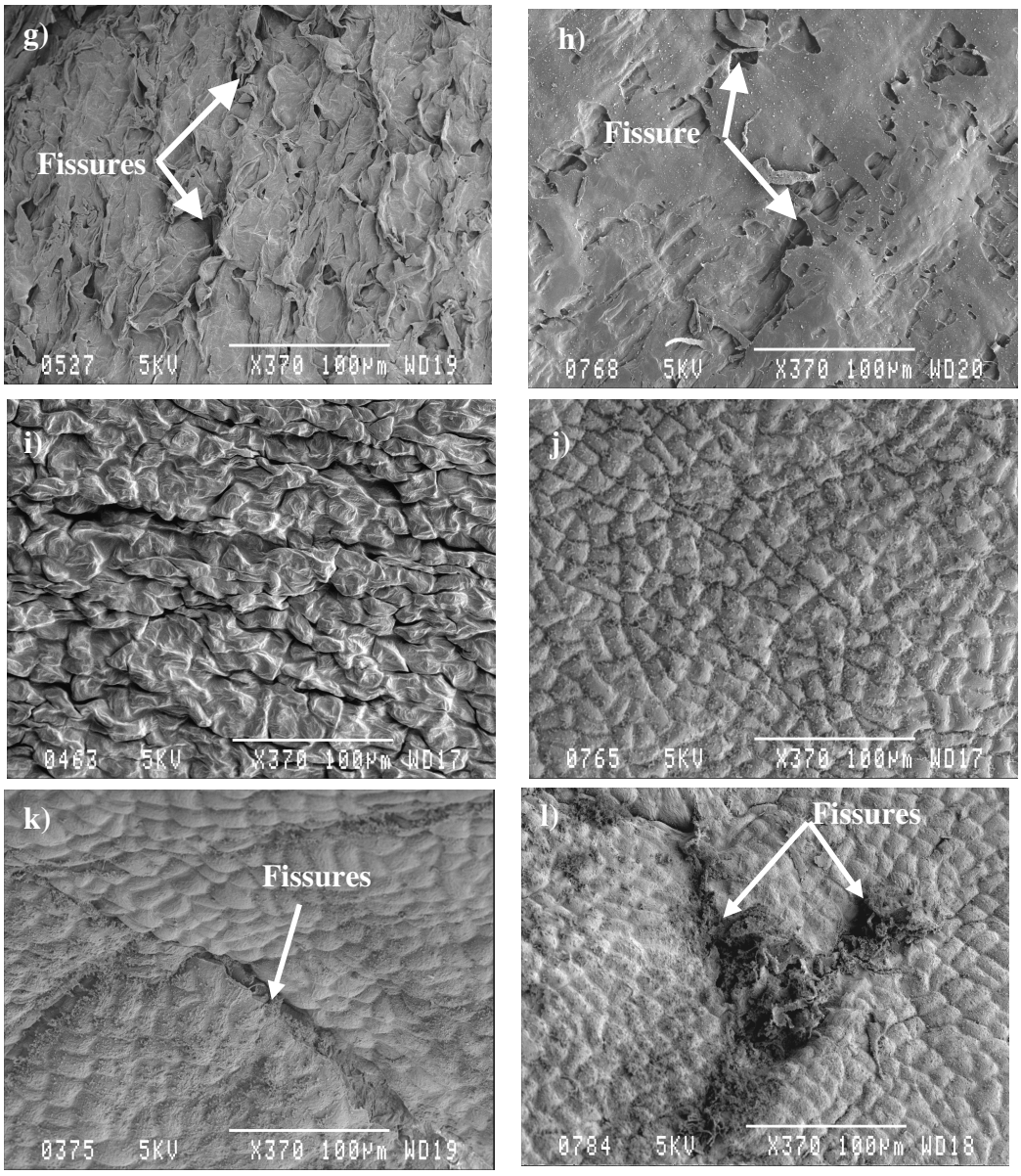

Figure 3.2.10 Continued

However, the seed coat is no longer intact in micronised (41 % moisture, 153 °C) cowpeas (Figures 3.2.10(c), 3.2.10(d), 3.2.10(g), 3.2.10(h), 3.2.10(k) and 3.2.10 (l)). Water has increased access into the cotyledon, which is fissured and has cavities (Figure 3.2.11). The presence of cavities and channels in the cotyledon was suggested by the decreased bulk density of micronised (41 % moisture, 153 °C) cowpeas (Table 3.2.1). Also, the contents of cotyledon cells are clumped together and detached from the cell wall, revealing spaces in most cells not filled with solids. Abdul-Kadir *et al.*

(1990) also reported significant increases in water absorption during 8 h of soaking (22 °C) of micronised (17 % moisture, 99, 107 °C) Pinto beans.

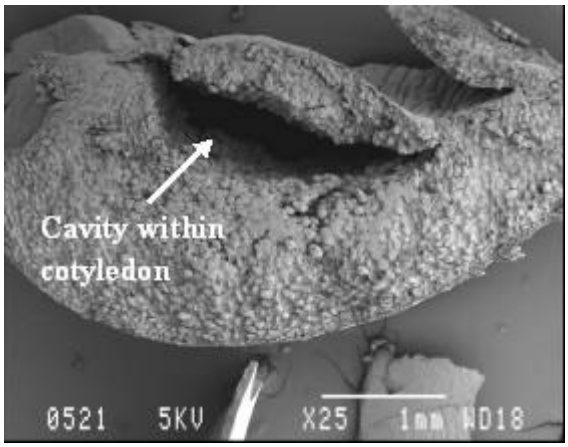

Figure 3.2.11 Formation of a cavity in the cotyledon of micronised (41 % moisture, 153 °C) cowpea seed

The hydration capacity of hydrothermally treated cowpeas was reduced by 20-25 % compared to unmicronised cowpeas (Table 3.2.1). Micronisation has been shown to reduce the hydrophilicity of legume proteins owing to unfolding of protein molecules thereby exposing hydrophobic sites (Zheng *et al*., 1998). Significant reductions in protein solubility for micronised (41 % moisture, 153 °C) cowpea seeds were observed in this study (Table 3.2.1). Cowpea protein from untreated seeds is highly hydrophilic (Mwasaru *et al*., 1999a) and contributes to water imbibition during soaking of cowpeas (Sefa Dedeh & Stanley, 1979b). The reduction in hydration capacity was possibly not due to shrinkage of stomata-like cells as was suggested by Fasina *et al*. (1999) for soaked, micronised barley.

3.2.3.4 Effect of hydrothermal treatment on cooking characteristics

Micronisation (41 % moisture, 153 °C) significantly ($P \leq 0.05$) reduced cooking time of the two cowpea varieties (Table 3.2.1). Cooking time was reduced by 44 % for Bechuana white cowpeas and 36 % for Var. 462 cowpeas. The reduction in cooking time concurs with the results on texture (work to cut through a seed) of the cooked cowpeas. For example, micronised (41 % moisture, 153 °C) Bechuana white cowpeas

required 4.7 N mm to cut through the seeds after approximately 30 min of cooking. Unmicronised Bechuana white cowpeas required 3.2 N mm to cut through the seeds after 60 min cooking. Cooked Bechuana white cowpeas were softer (required less work to cut) than cooked Var. 462 cowpeas. This means that the Var. 462 cowpeas yielded a harder texture (structure) after cooking with or without micronisation (41 % moisture, 153 °C), even though the seeds had lower density (Table 3.2.1).

Overall, the micronised (41 % moisture, 153 °C) cowpeas had a significantly softer texture than unmicronised cowpeas when cooked for the same length of time (Figure 3.2.4). Arntfield *et al*. (2001) indicated that micronised (33 % moisture, 138 and 170 °C) lentils required shorter time to reach acceptable hardness values for cooked lentils. The significant increase in enzyme-susceptible starch in micronised (41 % moisture, 153 °C) cowpeas (Table 3.2.1) probably contributed to the reduced cooking time. The microstructure of micronised (41 % moisture, 153 °C) cowpeas cooked for 15 min (half cooked stage) was similar to that of untreated cowpeas cooked for 30 min (half cooked stage) (Figure 3.2.5). Similarly, micronised (41 % moisture, 153 °C) samples attained a fully cooked microstructure after 30 min of cooking as compared with the 60 min required for untreated samples.

Since texture is an expression of structural, mechanical and surface properties of foods (Szczesniak, 2002), the loose and porous structure formed in some parts of the cotyledon during micronisation (41 % moisture, 153 °C) of cowpeas is weaker than the compact structure that is observed in unmicronised seeds (Figures 3.2.3(a); 3.2.3(b) & Figure 3.2.12), resulting in an overall softer texture in micronised (41 % moisture, 153 °C) seeds. This increased porosity of the cotyledon probably led to the higher rate of water absorption during both soaking and cooking of micronised (41 % moisture, 153 °C) cowpeas (Figures 3.2.2 & 3.2.7).

Comparison of SEM micrographs of moisture-conditioned Var. 462 and Bechuana white cowpeas micronised to 153 °C and soaked in water for 3 h (Figures 3.2.13(a) & 3.2.13(b)) shows marked cell separation along the middle lamella and the presence of cell wall remnants, which is similar to patterns reported in the cotyledon of lentils micronised to 138 °C (Arntfield *et al*., 2001) and in cooked cowpea cotyledon (Rockland & Jones, 1974; Sefa Dedeh *et al*., 1978).

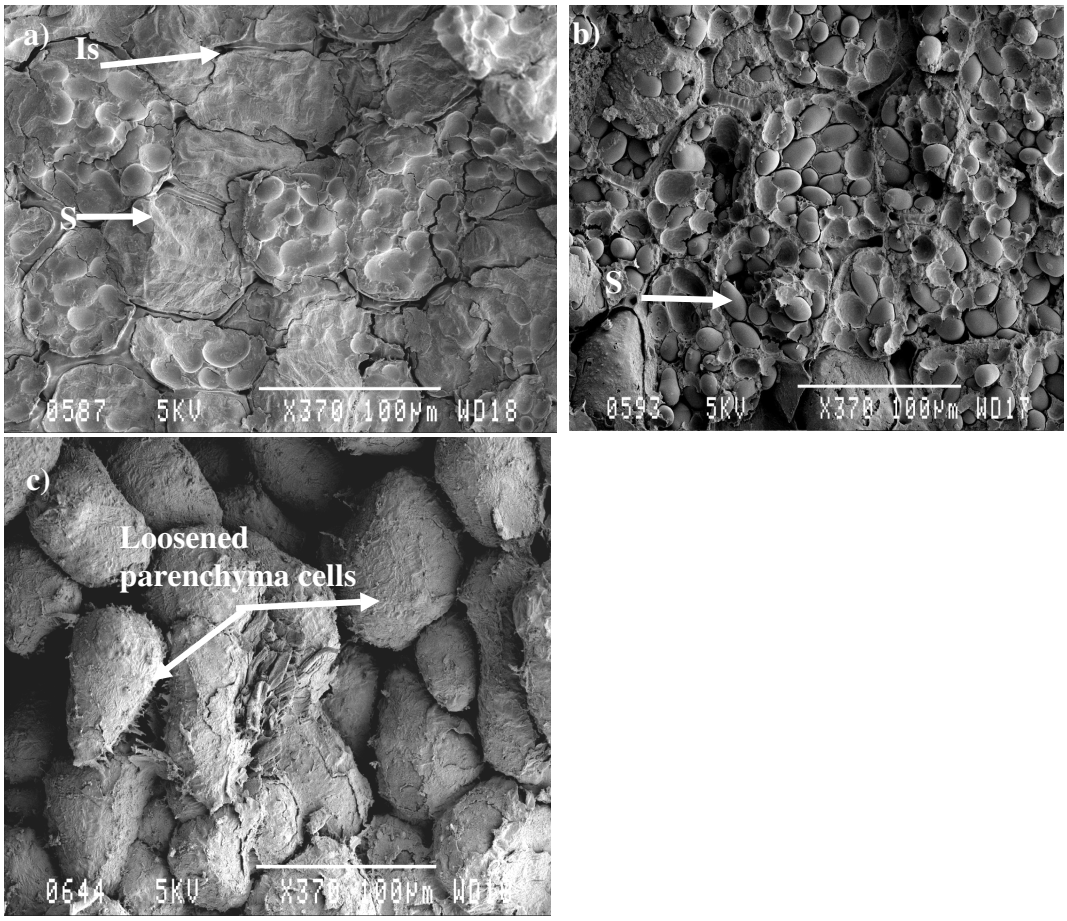

Figure 3.2.12 Cotyledon cross section of Bechuna white cowpea showing separation of parenchyma cells following micronisation; (a) unmicronised, (b) moisture-conditioned and (c) micronised (41 % moisture, 153 °C)

Cell separation could be an indication of solubilisation of pectic substances leading to the disintegration of the middle lamella (Rockland & Jones, 1974). These results might imply that micronisation to 153 °C solubilises or breaks down the pectic substances of the middle lamella into water-soluble fractions and contributes to shorter cooking times. This postulation was not supported by Arntfield *et al*. (2001) where a non-significant increase in soluble pectins was observed in lentils micronised to 138 and 170 °C.

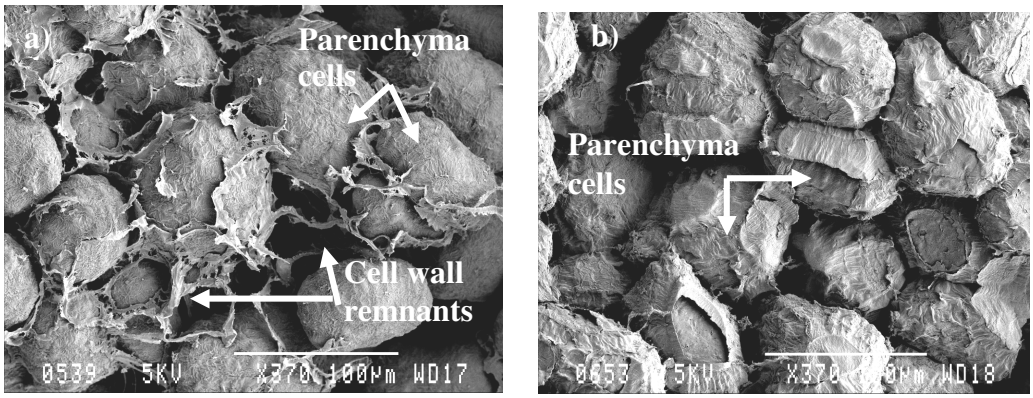

Figure 3.2.13 Varietal differences and micronisation (41 % moisture, 153 °C) effect on the cotyledon structure of two cowpea varieties: cotyledon cross section of micronised (41 % moisture, 153 °C) and soaked Var. 462 (a) and Bechuana white (b)

In addition, fissures that developed in the seed coat and cotyledon (Figures 3.2.9(c), 3.2.9(d), 3.2.9(g), 3.2.9(h), 3.2.9(k) 3.2.9(l) & 3.2.10) were instrumental in facilitating higher water uptake during cooking. Water uptake had a significant ($P \leq 0.001$) negative correlation (Var. 462, r = -0.82; Bechuana white, r = -0.92) with the texture of the cowpeas. Improved rates of water uptake during cooking of moisture-conditioned and micronised lentils (Cenkowski & Sosulski, 1997; Arntfield *et al.*, 2001) and split peas (Cenkowski & Sosulski, 1998) have also been reported.

3.2.3.5 Effect of micronisation (41 % moisture and 153 ºC) on splitting of cooked seeds

Micronisation (41 % moisture, 153 °C) significantly increased the tendency of both varieties to split during cooking, with the increase in splitting being more evident in Var. 462 cowpeas (Figure 3.2.8). This increase in splitting in micronised (41 % moisture, 153 °C) samples was probably due to the development of cracks in the testa and cotyledon during micronisation (Figure 3.2.9 and 3.2.10). The rapid temperature increase probably causes liquid water to turn into steam (gas). If the seed is able to hold the expanding gas, then pressure builds up and the cotyledon collapses under the pressure or explodes to release the pressure, resulting in modified cotyledon cells (Figures 3.2.3(c) & 3.2.3(d)).

3.2.4 Conclusions

The two cowpea varieties have similar cooking times, although Bechuana white has a softer cooked texture, absorbs more water during cooking and has a higher degree of splitting than Var. 462 cowpeas. This may be as a result of differences in cotyledon structure. The hydrothermal treatment significantly reduces the cooking time of both cowpeas, although differences in texture and splitting are still evident. Micronisation (41 % moisture, 153 °C) produces fissures in the cowpea seed coat and cotyledon, which leads to improvement in water uptake, a softer texture and increased splitting during cooking. The treatment also pregelatinises starch and denatures the protein. Cowpeas that are susceptible to splitting during cooking would not be appropriate for micronisation processing if whole cowpeas are desired after cooking.

3.3 Cowpeas cooking characteristics as affected by micronisation temperature: a study of the physicochemical and functional properties of starch[3]

Abstract

Moisture-conditioned (41 %) Bechuana white cowpeas were micronised to three temperatures (130, 153 and 170 °C). Cooking properties of the cowpea seeds and the role of starch-related properties were studied. Micronisation (41 % moisture, 130, 153 and 170 °C) in all cases significantly reduced the cooking time and thus the time required for the cowpea seeds to attain a suitably soft texture. This was attributed in part to the significant improvement in rate of water absorption during cooking and starch pregelatinisation as evidenced by loss of birefringence and increased susceptibility of the cowpeas starch to α-amylase digestion. However, micronisation of moisture-conditioned (41 % moisture) cowpeas to 170 °C resulted in a severe reduction in pasting properties of the cowpea flour possibly due to starch depolymerisation and/or amylose associated crosslinking. Due to these changes, M-170 °C cowpea seeds required a longer cooking time than the other two moisture-conditioned and micronised samples. Hence flour prepared from cowpeas treated at M-170 °C had less starch functionality.

Key words: cowpea, starch, micronisation temperature, gelatinisation, amylose,

[3] Accepted for publication in the *Journal of the Science of Food and Agriculture*

3.3.1 Introduction

Cowpeas are rich in starch and protein that provide relatively affordable protein and energy in carbohydrate-based diets of most developing countries (Giami, 2005). Cowpeas are consumed in different forms, one of which is in boiled stews (Taiwo, 1998), which is common in southern Africa. Dried cowpeas prepared in such a way require extended cooking times to attain a palatable texture and flavour that is acceptable to consumers. The cooking time (CT_{50}) of dried cowpeas ranges from 31 to 160 min (Akinyele *et al*., 1986), which translate into increased fuel energy use and time required. In most urban and peri-urban areas of Africa, fuel wood is the main source of energy and is in short supply.

Micronisation is one of the possible processes that could be used as a precooking treatment for cowpeas to decrease cooking time. According to Zarkadas and Wiseman (2001), micronisation refers to a short-time, high-temperature infrared processing method that utilises moisture and temperature to achieve conditions for optimum cooking and starch gelatinisation. As reported in section 3.2, micronisation of Bechuana white cowpeas (41 % moisture) to 153 °C reduced the cooking time by 44 %. Similar reductions in cooking time of moisture-conditioned and micronised legumes have been reported in Laird lentils (50 %) (Arntfield *et al*., 2001) and split peas (30 %) (Cenkowksi & Sosulski, 1998).

The effectiveness of micronisation of moisture conditioned legumes as a precooking treatment for legumes is dependent on several processing parameters. The final micronisation temperature is one of the critical process parameters during hydrothermal processing of leguminous seeds. It has been shown that after 15 min of cooking, lentils (33 % moisture) micronised to a high temperature (170 °C) coupled with lower final moisture content (7 %) had a significantly harder texture than lentils micronised (33 % moisture) to 138 °C (Arntfield *et al*., 2001) leading to increased cooking time to attain acceptable softness. This phenomenon impacts negatively on the effectiveness of the process and needs to be further investigated. Since starch is one of the major macromolecular constituents of cowpeas (Kerr *et al*., 2001), this research studied the effect of high (170 °C), medium (153 °C) and low (130 °C) micronisation temperatures on physicochemical and functional properties of cowpea

starch with the aim of explaining the possible differences in cooking characteristics of moisture-conditioned cowpeas micronised to higher temperatures.

3.3.2 Materials and methods

3.3.2.1 Raw materials

Bechuana white (cream colour) cowpeas (11.4 g per 100 seeds, 6mm by 5mm) supplied by Agricol (Potchefstroom, South Africa) were cleaned to remove chaff, shrivelled and broken seeds. The cleaned seeds were packed in propylene bags and stored at 4 °C until the time of use.

3.3.2.2 The hydrothermal process

The cowpeas were micronised according to the method described in section 3.2.2.2. Cowpea seeds were moisture conditioned to 41 % moisture (wb) by steeping in deionised water (1:5 w/v) for 6 h and holding for 12 h at 22 °C for the moisture to equilibrate throughout the seeds. The moisture-conditioned cowpeas were micronised in 160 g batches for 3, 6 and 8 min to final surface temperatures of 130 (M-130 °C), 153 (M-153 °C) and 170 °C (M-170 °C), respectively, using a tabletop microniser (Technilamp Pty, Johannesburg, South Africa). Following micronisation, the micronised cowpeas had 25 %, 12 % and 5.0 % moisture content for the M-130 °C, M-153 °C and M-170°C treatments, respectively. Figure 3.3.1 shows the experimental flow diagram. Cooking properties of the unmicronised and micronised (M-130 °C, M-153 °C and M-170 °C) samples were determined immediately. Since the cowpeas micronised to 130 °C had retained higher moisture content, these samples were freeze dried in preparation for milling.

3.3.2.3 Determination of moisture content

Moisture content of raw, tempered and micronised cowpea seeds was determined according to the method of Ajibola *et al*. (2003) as reported in section 3.1.2.1.

3.3.2.4 Determination of water absorption during soaking

Water absorption during soaking was determined according to a modified method of Agbo *et al*. (1987) as reported in section 3.1.2.4.

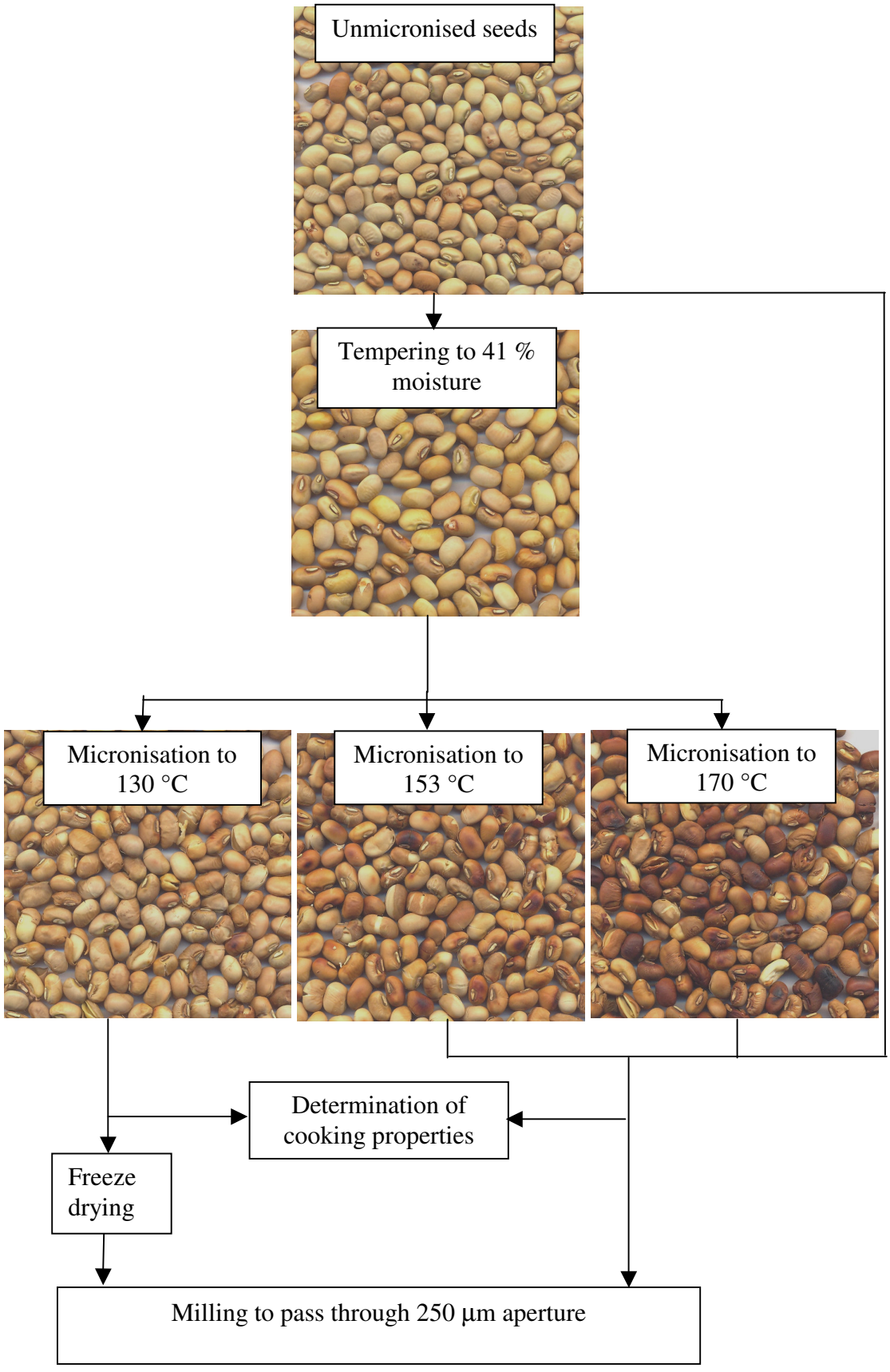

Figure 3.3.1 Experimental flow diagram

3.3.2.5 Determination of water absorption during cooking

The amount of water absorbed during cooking was determined according to a modified method of Cenkowski and Sosulski (1997) as reported in section 3.1.2.5.

3.3.2.6 Splitting of cowpea seeds during cooking

The tendency of seeds to split during cooking was determined according to the method of van Buren *et al*. (1986) as reported in section 3.1.2.6.

3.3.2.7 Texture of cowpea seeds during cooking

Texture was measured as work (area under the curve, Nmm) done to cut individual cowpea seeds through a distance of 5 mm at a speed of 4 mm/s based on the method of Sefa-Dedeh *et al*. (1978) as reported in section 3.2.2.11.

3.3.2.8 Determination of cooking time

A Mattson bean cooker (custom made) as described by Wang *et al*. (2003) was used to determine the cooking time of micronised (41 % moisture, 130, 153 and 170 °C) and unmicronised cowpea samples as reported in section 3.2.2.12.

3.3.2.9 Cowpea flour preparation

The cowpea samples (41 % moisture) micronised to 130 °C were freeze dried before milling. Raw and micronised (41 % moisture, 130, 153 and 170 °C) cowpeas were milled (Falling number mill, 3100) to pass through a 250 µm-aperture sieve. The milled flour samples were vacuum-sealed and stored at 4 ºC until they were used to determine total starch, enzyme-susceptible starch, amylose content, pasting and thermal properties.

3.3.2.10 Determination of total and enzyme-susceptible starch

Total starch content of the unmicronised and micronised (M-130 °C, M-153 °C and M-170 °C) cowpea flour was determined according to the AOAC method 996.11 using the Megazyme total starch assay kit (Megazyme, Wicklow, Ireland) (McCleary *et al*., 1997). Flour samples (0.1 g) of unmicronised and micronised (M-130 °C, M-153 °C and M-170 °C) cowpeas were vortexed in 0.2 ml aqueous ethanol (80 % v/v) and digested by 300 units of thermostable α-amylase (in 3 ml of 50 mM MOPS buffer, pH 7) in a boiling water bath (96 °C) for 6 min with intermittent stirring. The

samples were cooled and 4 ml of 200 mM sodium acetate buffer (pH 4.5) was added followed by 30 min digestion using 20 units of amyloglucosidase (0.1 ml) at 50 °C. The method was modified slightly to determine enzyme-susceptible starch (ESS) by digesting the samples with thermo stable α-amylase at 37 °C. The hydrolysed glucose was determined using the glucose oxidase peroxidase (GOPOD) assay.

3.3.2.11 Determination of digestible amylose

The digestible amylose content of the cowpea flour was determined according to the Con A method described by Gibson, Solah & McCleary, (1997) using the Megazyme amylose/amylopectin assay kit (Megazyme, Wicklow, Ireland).

3.3.2.12 Determination of carbohydrate solubility using size exclusion HPLC-Gel permeation chromatography

HPLC-Gel Permeation (HPLC-GP) chromatography was performed using three Shodex lonpax S-800 series styrenedivinylbenzene columns connected in series (Showa Denko K.K., Tokyo, Japan) (Jackson, Waniska & Rooney, 1989). The column packing was a strong sodium cation-exchange resin, designed for the separation of saccharides. The three columns were connected in the order of S-805/S (estimated exclusion limit (EEL) = 5 x 106 MW), S-804/S (EEL = 5 x 105 MW), and S-803/S (EEL = 5 x 104 MW). An oven maintained the column temperature at 50 ºC. The mobile phase was deionised water, HPLC-grade, which was pumped with a Beckman-110B HPLC pump operating at 1.0 ml min^{-1} (Beckman Instrument Inc., Berkeley, CA.). The columns were connected to a Waters model 410 refractive index detector (Millipore Corporation, Milford, MA). The refraction index cell was maintained at 50 ºC. Pullulan standards (MW 5300-853000) were utilised to identify the molecular weight (MW) of the eluant. Soluble amylopectin, amylose, oligosaccharides and sugars of unmicronised and M-170 °C flour were extracted in water for 10 min at 98 °C and quantified in the HPLC-GP system.

3.3.2.13 Enzyme treatment and light microscopy of cowpea flour dispersions

Enzyme digestion of cowpea flour was conducted in order to identify the different macromolecules involved in reduced pasting viscosity of the M-170 °C cowpea flour. The digestion process was based on the method reported by Zhou, Hoover & Liu (2004). Cowpea flour was sequentially digested with α-amylase followed by protease

and the remaining undigested material was split in half; one half was digested with α- amylase and the other part with pectinase. Cowpea flour (0.6 g) was mixed with 4 ml α-amylase (Megazyme International, Wicklow, Ireland) in 30 ml pH 7 MOPS (3-N-Morpholinopropanesulphonic acid) buffer and digested at 37 ºC for 16 h. The slurry was centrifuged 1000 *x g* for 5 min and the supernatant was discarded. The sediment was washed in 15 ml of water followed by centrifugation. A sample of the sediment was taken and mounted on a glass slide and protein was stained for 5 min with 1 drop of Acid Fuchsin (1 % w/v in water; Fisher Scientific, USA) while Congo Red (1 % w/v in water; Fisher Scientific, USA) stained for glucans. The remaining sediment was digested with 10 ml protease (1g/ml, Sigma EC 232-752-2) in pH 7.5 phosphate buffer for 1 h at 37 °C. The sample was centrifuged and the supernatant was discarded followed by washing with water. A sample of the sediment was mounted on a glass slide as described before. The remaining sediment was split into two fractions. One fraction was digested for 1 h in 10 ml α-amylase solution. The pH of the remaining fraction was adjusted to 4.5 using 25 % acetic acid followed by 1 h digestion with 2 ml of pectinase (Pectinase from Rhizopus Sp, CAS No. 9032-75-1, Sigma Inc, St Louis Missouri) at room temperature. The remaining sediments were mounted on glass slides. Photomicrographs of the mounted samples were taken using a Nikon Coolpix 995 camera (Nikon USA) mounted on a Zeiss Universal bright field microscope equipped with polarising filters. Samples were viewed with polarisers to determine the extent of birefringence and without polarisers to view the extent of staining for components.

3.3.2.14 Isolation of starch from micronised (41 % moisture, 130 °C and 170 °C) cowpeas

Starch from unmicronised and micronised (41 % moisture, 130 °C and 170 °C) cowpeas was isolated based on the method of Taylor, Novellie & Liebenberg (1984). Cowpea starch was isolated from raw and micronised (41 % moisture, 130 and 170 °C) cowpeas milled to pass through a sieve with aperture size 250 µm. The flour (100 g) was mixed with 500 ml deionised water and stirred at 22 °C for 2 h. The slurry was wet milled using a Retsch wet mill (Monitoring and Control Laboratories, Johannesburg, South Africa) and filtered through sieves (Labotech, Johannesburg, South Africa) with aperture sizes 212, 108, 75 and 45 µm on to a collecting pan. The sediment on the sieve was suspended in distilled water and wet milled and the

resulting slurry filtered through the sieves. The wet milling process was repeated until the filtrate was clear. The filtrate was centrifuged at 3880-x *g* for 5 min (15 °C). The supernatant was carefully decanted and the sediment was re-suspended in deionised water and vigorously mixed. The mixture was centrifuged at 3880 x *g* for 5 min (15 °C) and the supernatant liquid was decanted carefully. The brown layer on top of the starch sediment was carefully scooped out. The remaining starch sediment was re-suspended in deionised water and centrifuged. The washing, removing of the brown layer, suspension in water and centrifuging was repeated for 6 to 7 times. The starch was then washed with hexane to remove fat residues followed by drying at 37 °C for 48 h. The dried starch was ground with a motor and pestle to pass through a sieve with aperture size 109 µm.

3.3.2.15 Thermal analysis of cowpea flour and extracted starch

Thermal properties of flour from unmicronised and micronised (41 % moisture, 130 and 170 °C) cowpea seeds were measured according to the method reported by Ji, Seetharaman, Wong, Pollack, Duvick, Jane & White (2003). Cowpea flour samples were mixed with water in the ratio of 2:1 and heated in a sealed stainless steel pan. A Perkin-Elmer DSC-7 analyzer (Norwalk, CT) equipped with thermal analysis software (Perkin-Elmer Corporation, Norwalk, CT) was used to scan the samples at a rate of 10 ºC min^{-1} from 30 to 110 ºC. All measurements were carried out on duplicate samples. The following thermal parameters were measured: the melting enthalpy (Δ H) in J g^{-1}, peak onset (To), peak (Tp) and peak end (Tc) temperatures. The degree of starch conversion was calculated as: 100 - % decrease in enthalpy as a result of the heat treatment.

3.3.2.16 Pasting properties of cowpea flour and isolated starch

Pasting properties of cowpea flour and starch isolated from unmicronised and micronised (41 % moisture, 130 and 170 °C) samples were determined using a rapid visco-analyser's (Newport Scientific Pty Ltd, Warriewood, Australia) standard profile 2 with modifications to allow for lower boiling point (96 °C). Suspensions of flour (3.5 g) on a dry basis were prepared in 25 ml of deionised water. The suspension was equilibrated at 50 ºC for 1 min followed by programmed heating to 91 ºC at a uniform rate of 5 ºC min^{-1} with constant stirring at 160 rpm. The heated slurry was held at

91 ºC for 7 min then cooled to 50 ºC at the same rate and held at this temperature for 2 min.

3.3.2.17 Statistical analysis

The experimental assays were repeated three times to generate data that was analysed using Statistica (StatSoft, Inc., Tulsa, OK) statistical software version 6.0. Analysis of variance (ANOVA) was used to evaluate the data based on a 5 % level of significance. When the F-test was significant, differences between means were determined using the least significant difference (LSD) test.

3.3.3 Results and discussion

Following the micronisation process (41 % moisture and Infrared heating), the M-130 °C cowpeas had significantly ($P \leq 0.05$) higher moisture content than the unmicronised and the other micronisation temperatures (Table 3.3.1). As such, cowpeas micronised to 130 °C would require additional drying to achieve shelf stability (Table 3.3.1). Increasing the micronisation temperature to 153 and 170 ºC reduced the moisture content to levels adequate for shelf stability.

Cooking of dry leguminous seeds involves seed hydration and heating. Dry seeds are sometimes hydrated by soaking in water prior to cooking. The length of soaking time depends on hydration properties of the seeds (Sefa-Dedeh *et al*., (1978); Sefa-Dedeh & Stanley (1979a). Micronised (41 % moisture, 130, 153 and 170 ºC) cowpeas had a higher rate of water absorption and absorbed more ($P \leq 0.05$) water during 6 h of soaking at 22 ºC than the unmicronised cowpeas (Figure 3.3.2). Water absorption during soaking of legume seeds is related to physical and chemical properties of the seed, such as seed coat properties, starch, protein (Sefa-Dedeh & Stanley, 1979a), and density as explained in section 3.2.3.1. It was observed that micronised (41 % moisture, 153 °C) cowpea seeds were less dense than unmicronised cowpea seeds and showed fissured seed coat and cotyledon, which contributed towards increased water uptake during soaking.

Following 3 h of soaking, M-153 °C and M-170 °C cowpeas absorbed significantly ($P \leq 0.05$) more water than the M-130 °C cowpeas (Figure 3.3.2). The difference in water absorption pattern during soaking (22 °C) was probably due to the differences in

moisture content following the micronisation treatment (Table 3.3.1). A positive correlation has been reported between the initial moisture content of legume seeds and the rate of water uptake during the 1st hour of soaking (Moscoso, Bourne & Hood, 1984).

Table 3.3.1 Effect of micronisation temperature (130, 153 and 170 °C) on some physicochemical characteristics of moisture-conditioned (41 %) cowpeas

Physicochemical characteristic	[y]Unmicronised	[z]Micronisation temperature (°C)		
		130	153	170
Moisture (g kg^{-1})	89^{c} (4)	252^{a} (18)	121^{b} (22)	50^{d}(9)
Cooking time (min)	57^{a} (2)	30^{c} (5)	32^{c} (7)	39^{b} (2)
Water absorbed (g kg^{-1})				
30 min of cooking	875^{d} (45)	1189^{c} (36)	1393^{b} (45)	1487^{a} (37)
60 min of cooking	1538^{b} (31)	1419^{c} (39)	1713^{a} (39)	1667^{a} (50)
Texture (Work) (Nmm)				
30 min of cooking	6.9^{a} (1.6)	4.4^{b} (1.0)	4.7^{b} (1.1)	4.4^{b} (0.9)
60 min of cooking	3.2^{a} (0.9)	3.1^{a} (0.8)	2.6^{b} (0.9)	3.0^{a} (0.4)
Splits (%)				
30 min of cooking	3^{b} (1.6)	40^{a} (8.4)	44^{a} (7.1)	42^{a} (1.6)
60 min of cooking	45^{c} (10.0)	74^{a} (10.1)	62^{b} (7.6)	48^{c} (4.6)
Total starch (g kg^{-1})	445 (17.2)	452 (13.3)	459 (17.3)	473 (15.6)
ESS (%)	21^{c} (2.7)	73^{a} (2.8)	68^{b} (2.8)	68^{b} (1.8)
Digestible amylose (%)	19.5^{a} (1.8)	12.7^{b} (3.3)	15.1^{b} (2.3)	14.9^{b} (1.7)

ESS= Enzyme-susceptible starch; [y]Unmicronised = Raw, [z]Micronisation temperature (°C) = Temperature for micronisation of moisture-conditioned seeds (41 %); means followed by the same letter within a row are not significantly different at level $P \leq 0.05$; standard deviations of the means are in parenthesis

3.3.3.1 Cooking characteristics of cowpeas

Cooking time is one of the cooking characteristics of leguminous grains that is widely evaluated, since long cooking time seems to be one of the main factors contributing to the dismal utilisation of legumes such as cowpeas (Taiwo, 1998).

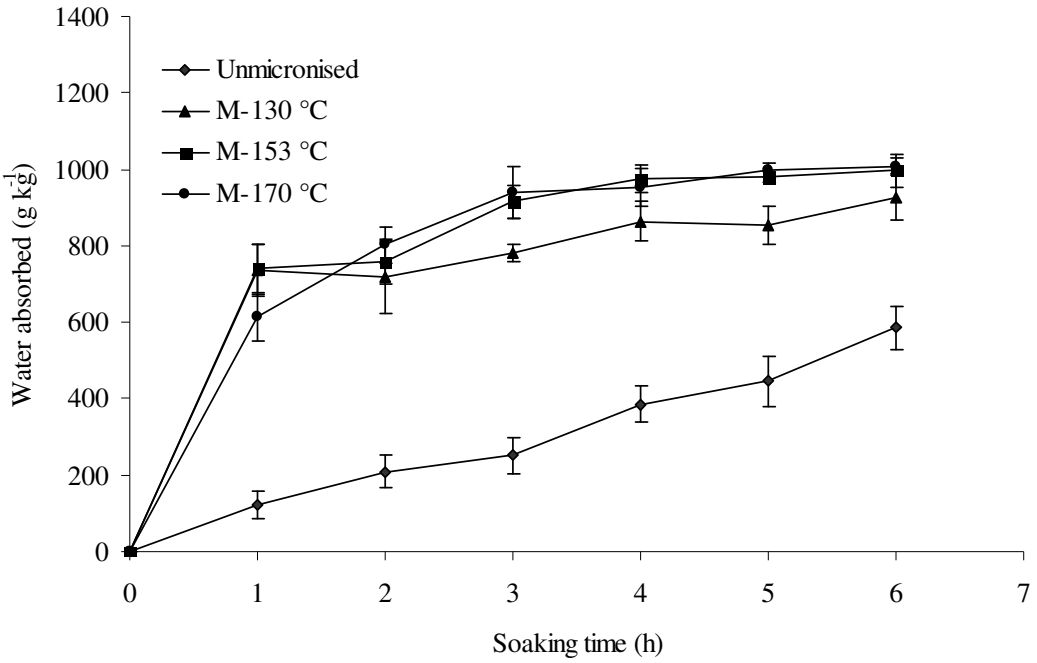

Figure 3.3.2 Effect of micronisation temperature (130 °C, 153 °C and 170 °C) on water absorption during 6 h of soaking (22 °C) Bechuana white cowpeas (vertical bars indicate standard deviations of the means Unmicronised= Raw, M= Micronised (41 % moisture, 130 °C, 153 °C and 170 °C))

The unmicronised Bechuana white cowpeas had a cooking time of 57 min, which was significantly ($P \leq 0.05$), reduced by 47, 44 and 32 % following micronisation (41 % moisture-conditioning and infrared heating) to 130, 153 and 170 °C, respectively (Table 3.3.1). Similar reductions in cooking time of moisture-conditioned and micronised legumes have been reported for lentils (Arntfield *et al*., 1997; Cenkowski & Sosulski, 1997; Arntfield *et al*., 2001), split peas (Cenkowski & Sosulski, 1998) and beans (Bellido *et al*., 2006). Cooking time in legumes refers to the time required

for seeds to attain a texture that is acceptable for consumption (Arntfield *et al*., 2001). It is evident that unmicronised cowpeas required a significantly ($P \leq 0.05$) longer cooking time than the three micronised (41 % moisture, 130, 153 and 170 °C) samples in order to attain a soft texture (Figure 3.3.3; Table 3.3.1). This is consistent with other research work on moisture-conditioned and micronised legumes (Arntfield *et al*., 1997; Cenkowski & Sosulski, 1997; Arntfield *et al*., 2001; Bellido *et al*., 2006). The softening of dry legume seed texture during cooking has mainly been ascribed to parenchyma cell separation along the middle lamella (Sefa-Dedeh *et al*., 1978) due to β elimination of pectic substances (Liu *et al*., 1993b; Coultate, 2002) and starch gelatinisation within the cotyledon parenchyma cell (Sefa-Dedeh *et al*., 1979).

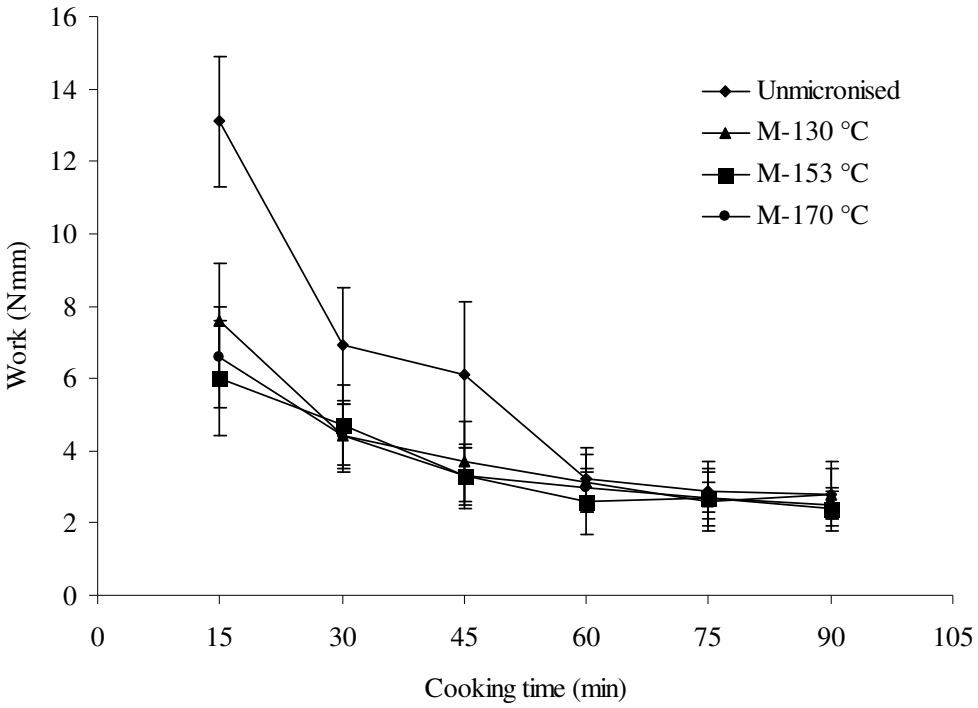

Figure 3.3.3 Effect of micronisation temperature (130 °C, 153 °C and 170 °C) on texture (Work, N mm) during 90 min of cooking Bechuana white cowpeas (vertical bars indicate standard deviations of the means Unmicronised= Raw, M= Micronised (41 % moisture, 130 °C, 153 °C and 170 °C))

Since β elimination of pectic substances and starch gelatinisation require water, improved water uptake during cooking of legumes has been related with reduction in cooking time (Cenkowski & Sosulski, 1997; Phadi, 2004). A significant ($P \leq 0.05$) negative correlation ($r = -0.90$) existed between water absorption during cooking and texture (work required to cut through a seed). Micronisation (41 % moisture, 130, 153 and 170 °C) significantly improved the amount of water absorbed by the cowpeas during the first 45 min of cooking (Table 3.3.1, Figure 3.3.4) possibly due to reduced bulk density and development of fissures as reported in section 3.2.3.2.

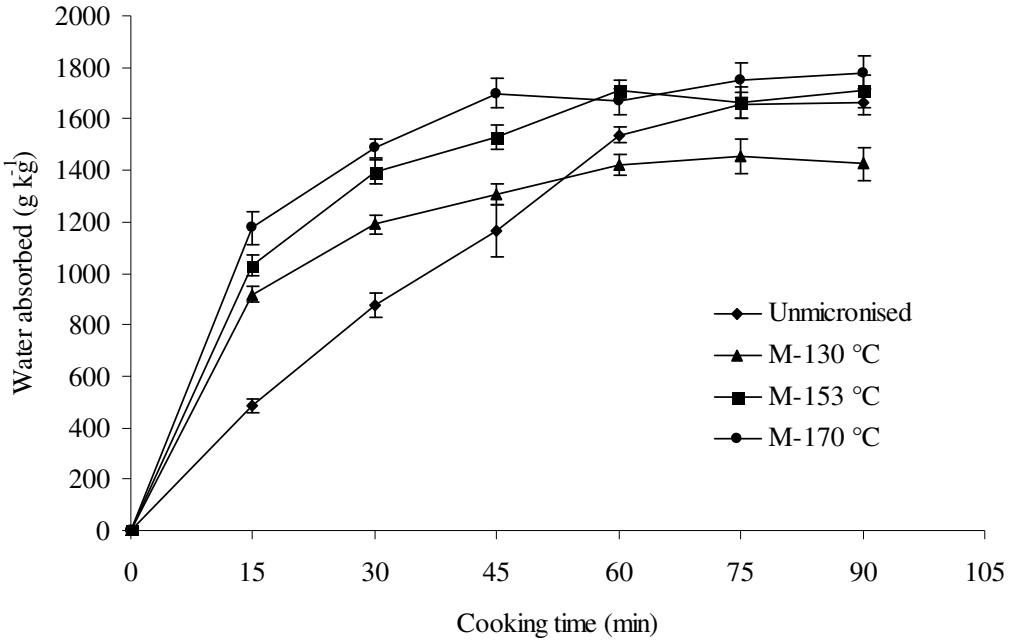

Figure 3.3.4 Effect of micronisation temperature (130 °C, 153 °C and 170 °C) on water absorption during 90 min of cooking Bechuana white cowpeas; (vertical bars indicate standard deviations of the means Unmicronised= Raw, M= Micronised (41 % moisture, 130 °C, 153 °C and 170 °C))

Micronised (41 % moisture, 153 °C) cowpea seeds as reported in Section 3.2 to have reduced bulk density in comparison to unmicronised seeds. The reduction in bulk

density implies that the micronised (41 % moisture, 130, 153 and 170 °C) seeds have air spaces within the cotyledon, which would enhance water uptake.

Pressure build up within the seeds during micronisation gives rise to the development of fissures in the seed coat, cotyledon and cell wall of micronised (41 % moisture, 153 °C) cowpeas seeds. These fissures contribute towards improved water absorption by the seeds during cooking. In addition, the fissuring of the cotyledon may also have led to significant ($P \leq 0.05$) increase in the incidence of splitting in micronised (130, 153 and 170 °C) cowpeas when cooked for 30 min (Table 3.3.1, Figure 3.3.5). The level of splitting in micronised (41 % moisture, 130, 153 and 170 °C) samples at 30 min of cooking was not significantly ($P \leq 0.05$) different to the level of splitting for unmicronised cowpeas cooked for 60 min. Since the cooking time of M-130 °C, M-153 °C and M-170 °C cowpeas was 30, 32 and 39 min respectively (Table 3.3.1), it means that effectively there is no difference in splitting of the cooked samples.

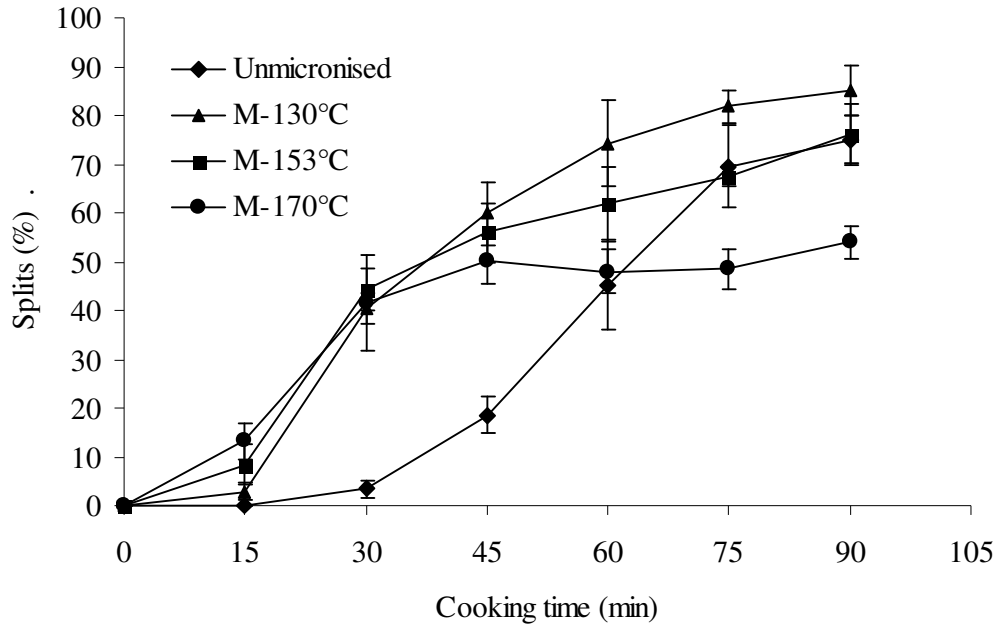

Figure 3.3.5 Effect of micronisation temperature (130 °C, 153 °C and 170 °C) on splitting (%) during 90 min of cooking Bechuana white cowpeas, (vertical bars indicate standard deviations of the means Unmicronised= Raw, M= Micronised (41 % moisture, 130 °C, 153 °C and 170 °C))

Splitting of unmicronised, M-130 ºC and M-153 °C cowpeas increased with extended cooking time, while there was no significant increase in splitting after 30 min of cooking M-170 °C cowpeas. It has been postulated that the incidence of splitting during cooking of unmicronised cowpea seeds is positively related to increase in water absorption during cooking (Taiwo, 1998). Although the M-170 °C cowpeas had high water absorption throughout the cooking process comparable with unmicronised, M-130 °C and 153 °C cowpeas, this was not accompanied with increased incidence of splitting during extended cooking. This reduction in splitting during extended cooking shows that micronisation to a higher temperature possibly resulted in a cotyledon structure that did not easily disintegrate during extended cooking.

Overall, the two lower micronisation temperatures (130 and 153 °C) were more effective in reducing cooking time of cowpeas than the higher temperature of 170 °C (Table 3.3.1). The longer ($P \leq 0.05$) cooking time and splitting pattern observed in M-170 °C cowpeas could possibly be due to differences in the extent of starch modification during micronisation involving higher temperatures.

3.3.3.2 Effect of micronisation temperature of cowpea seeds on starch-related properties

Since there were no significant differences between the cooking characteristics of M-130 °C and M-153 °C cowpeas, some of the subsequent work on starch was conducted using M-130 °C and M-170 °C cowpeas to represent a mild and intense heat treatment. Starch gelatinisation is a complex process, where the structure and functionality of the end product varies depending on process conditions such as temperature, moisture as well as the presence or the absence of shear (Tananuwong & Reid, 2004; van de Einde, Akkermans, van der Goot & Boom, 2004). The micronisation (130 and 170 °C) of cowpeas with 41 % moisture changed the crystalline order and nature of the cowpea starch granules. This is evidenced by reduced transitional enthalpies (Table 3.3.2), loss of birefringence and increased susceptibility to α-amylase digestion (Table 3.3.1).

Cowpea flour milled from cowpeas micronised to 130 and 170 °C seeds had higher transition onset (To) and peak (Tp) temperatures (Table 3.3.2). Enthalpies of cowpea flours were much lower than that of the starch isolated from the samples; however,

thermal transition temperatures of the isolated starch were lower compared to flour samples (Table 3.3.2). Transitional enthalpies for heterogeneous systems such as cowpeas flour are a composite of energy transitions in the system, which includes starch gelatinisation and protein denaturation (Henshaw *et al*., 2003). The starch isolated from micronised (41 % moisture, 130 and 170 °C) samples also exhibited higher gelatinisation and onset temperatures (To). Similar thermal transitions for cowpea flour have been reported earlier (Henshaw *et al*., 2003).

Table 3.3.2 Effect of micronisation temperature on thermal properties of cowpea flour and isolated starch

Thermal properties	[y]Unmicronised	[z]Micronisation temperature (°C)		
		130	153	170
Cowpea flour				
Onset To (ºC)	70.5[c] (0.1)	74.8[b] (1.2)	78.9 [a] (0.9)	74.4[b] (0.4)
Peak (ºC)	78.0[c] (1.0)	80.8 [bc] (0.2)	83.6[ab] (2.1)	84.4[a] (0.4)
Tc (ºC)	85.7 [b] (1.4)	93.5[ab] (2.2)	94.3[a] (7.9)	95.6 [a] (3.9)
Enthalpy (Δ H, J/g sample)	2.5 (0.6)	4.02 (2.1)	2.2	1.8 (0.8)
Cowpea starch				
Isolated starch yield (%)	66	44	ND	11
Onset To (ºC)	62.7 [b] (0.6)	68.0 [a] (1.3)	ND	69.8 [a] (0.3)
Peak (ºC)	72.7 [b] (0.4)	76.4 [a] (0.2)	ND	77.3 [a] (0.7)
Tc (ºC)	87.3 (3.3)	97.2 (4.7)	ND	93.7 (2.1)
Enthalpy (Δ H, J/g)	12.9 (1.8)	11.9 (4.0)	ND	5.5 (0.6)
Change in enthalpy (%)		8	ND	57

[y]Unmicronised = Raw, [z]Micronisation temperature (°C) = Temperature for micronisation of moisture-conditioned seeds (41 %); Means followed by the same letter within a row are not significantly different at level $P \leq 0.05$; standard deviations of the means are in parenthesis; ND = Not determined

The higher enthalpies observed in starch isolated from micronised (41 % moisture, 130 and 170 °C) samples could possibly be due to non-homogeneous thermal treatment among cowpeas and/or within the cowpea which was characterised by the reduced yields of isolated starch from micronised (41 % moisture, 130, and 170 °C) cowpeas. This contributed to the higher standard deviations in transitional enthalpies for these samples (Table 3.3.2). When the starch granules from the micronised (41 % moisture, 130 and 170 °C) seeds were observed under polarised light (Figure 3.3.6 (2b) and 3.3.6 (3b)), they did not exhibit birefringence.

Light microscopy of α-amylase digested flours from unmicronised and micronised (41 % moisture, 130 and 170 °C) cowpeas revealed a reduction in the amount of starch granules in micronised (41 % moisture, 130 and 170 °C) samples. This confirmed that the modified starch granules from micronised seeds (41 % moisture, 130 and 170 °C) were more susceptible to α-amylase digestion (Table 3.3.1) than native starch granules of unmicronised flour. These results indicate that micronisation of moisture-conditioned cowpea seeds altered the granular structure of cowpea starch thereby requiring less time to hydrate and disrupt the remaining starch structure during subsequent cooking. Cenkowski and Sosulski (1997) used DSC analysis of lentil flours, to demonstrate that 5 min of cooking was adequate to fully gelatinise the starch in micronised (38.6 % moisture, 150 °C) lentils as compared to 50 % of the starch for untreated lentils. Gelatinisation of starch during cooking of legumes is a very important phenomenon that has a positive correlation with texture of cooked seeds (Arntfield *et al*., 1997).

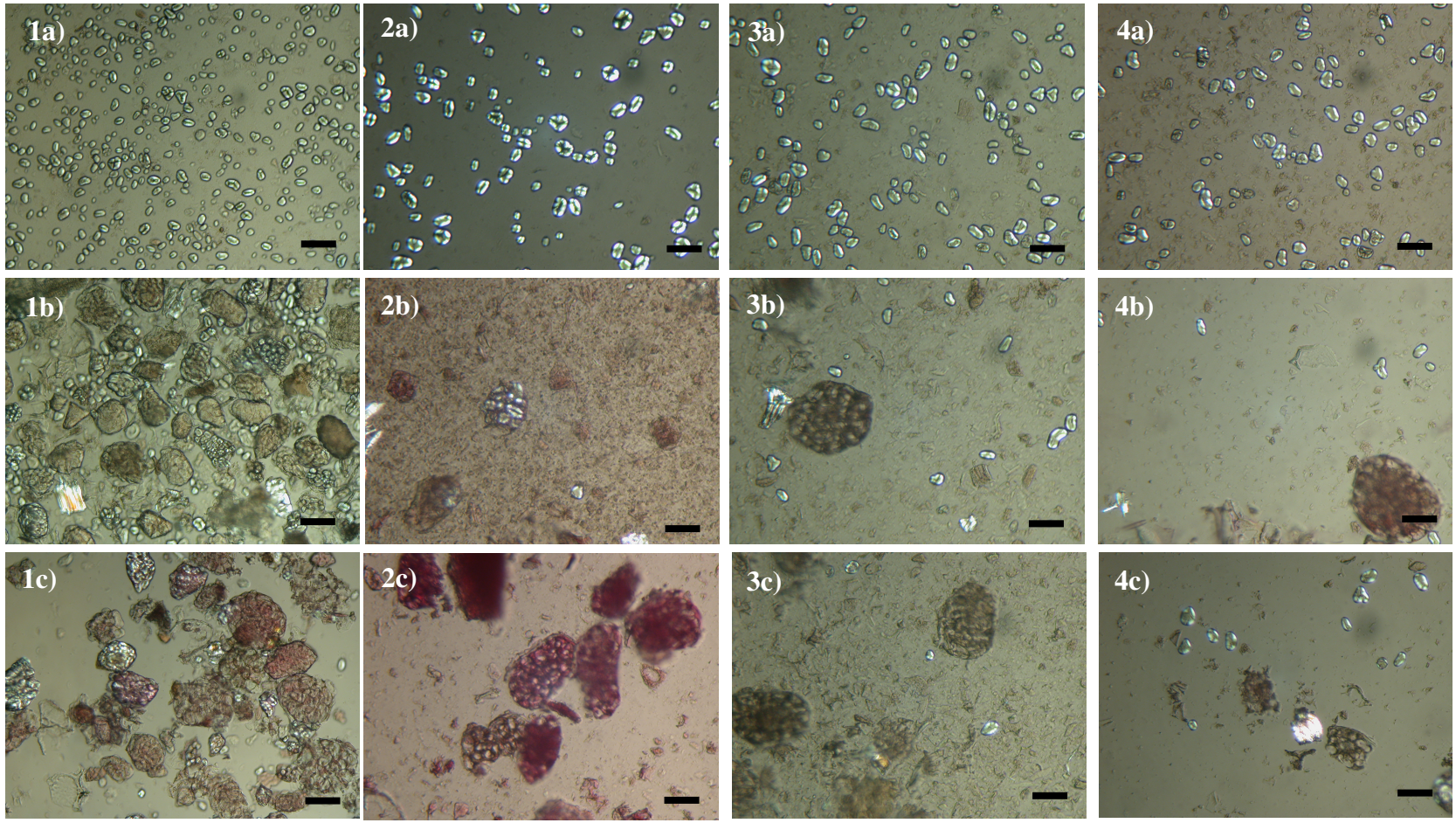

Figure 3.3.6 **Effect of micronisation temperatures on cowpea flour from micronised (41 % moisture, 130 °C and 170°C) seeds during sequential enzyme digestion (stained with acid Fuchsin): 1 = No enzyme treatment, 2 = α –amylase, 3 = α –amylase - protease, and 4 = α –amylase-protease- α –amylase; a = unmicronised, b = M-130 ºC and c = M-170 ºC, Bar ~50 nm**

Pasting curves have been used to track starch granule swelling and stability of native and hydrothermally treated cowpea flours (Prinyawiwatkul *et al*., 1997c; Henshaw *et al*., 2002). The flour from unmicronised cowpeas exhibited a characteristic pasting curve (Figure 3.3.7), which was comparable with patterns reported for cowpea flours (Prinyawiwatkul *et al*., 1997c; Henshaw *et al*., 2002). However, flour from micronised seeds (41 % moisture, 130 and 170 °C) did not give the characteristic cold swelling peak expected in pregelatinised starch (Lai, 2001) (Figure 3.3.7).

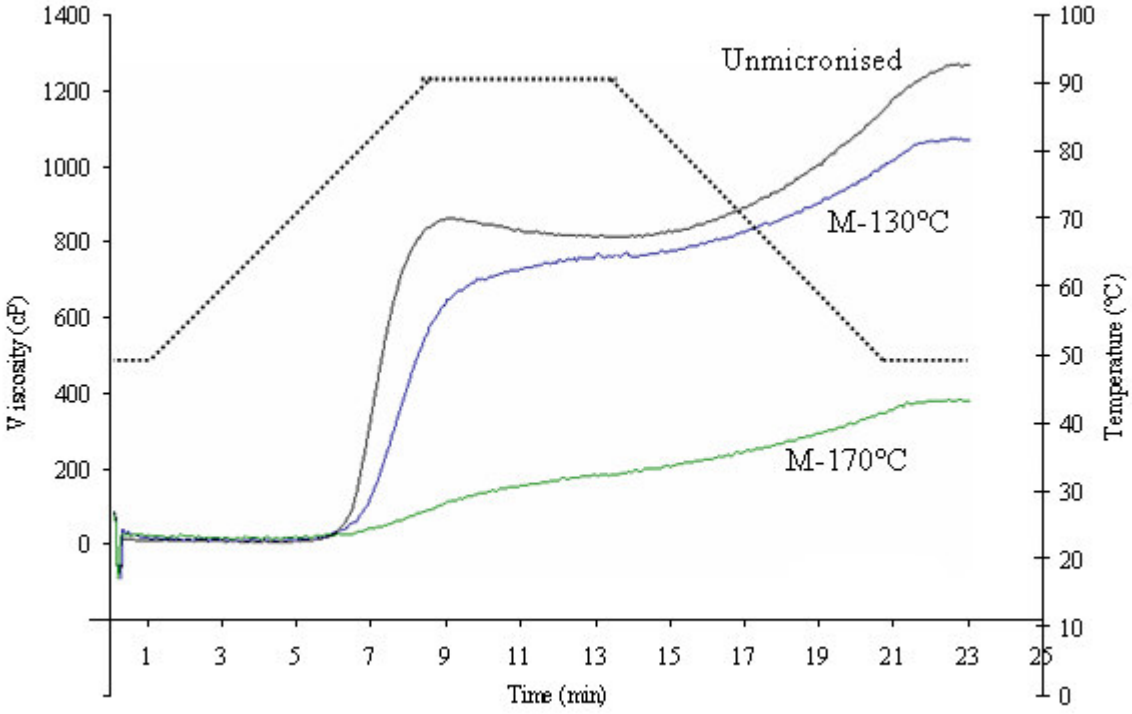

Figure 3.3.7 **Effect of micronisation temperature on pasting properties of cowpea flour from micronised (41 % moisture, 130 °C and 170 °C) seeds, (Unmicronised= Raw, M= Micronised (41 % moisture, 130 and 170 °C))**

Some of the starch granules were imbedded in the denatured protein matrix within the parenchyma cells (Figures 3.3.6 (2a) and 3.3.6 (3a)), which could reduce water access. Similarly, Cenkowski and Sosulski (1997) indicated the absence of a cold swelling peak for flour from micronised (26 % moisture; 110 °C) split peas. Prinyawiwatkul *et al.* (1997c) also illustrated that flour from cowpeas that had been soaked (24 h) and boiled (45 min) did not exhibit cold swelling peak.

The pasting curve for the M-130 °C flour shifted towards higher temperatures ($P \leq 0.05$) (Figure 3.3.7). Similar results have also been reported in microwave treated lentil starch (Gonzalez & Perez, 2002). Increase in pasting temperature of hydrothermally treated starch has been related to granule modification that limits water access into the granule (Hoover & Manuel, 1996a) and the presence of other competing hydrophilic molecules such as protein. The increase in pasting temperature is consistent with results on thermal properties of the cowpea flours which showed a 4 °C increase in transition onset temperature (To) for the M-130 ºC cowpea flour (Table 3.3.2). In hydrothermally treated starches, increase in onset temperature (To) is mainly attributed to amylose-amylose interactions that lead to increased starch granule crystallinity requiring higher temperatures to be melted (Hoover & Manuel, 1996a; Hoover & Manuel, 1996b). Concomitantly, micronisation (41 % moisture, 130 °C and 170 °C) significantly reduced the amount of digestible amylose of the cowpea flour (Table 3.3.1). Thus the reduction in digestible amylose could possibly be due to the formation of amylose-lipid complexes (Hoover & Manuel, 1996a; Hoover & Manuel, 1996b) and/or re-crystallisation of dispersed amylose during retrogradation of gelatinised starch (Arntfield *et al.*, 1997).

In addition there was a significant ($P \leq 0.05$) progressive decline in pasting viscosities of the flours with increase in micronisation temperature (Figure 3.3.7). The flour from M-130 ºC cowpeas had lower ($P \leq 0.05$) peak viscosity than the flour from unmicronised seeds, while there was a severe ($P \leq 0.05$) reduction in flour paste viscosity for the M-170 ºC flour (Figure 3.3.7). This is in contrast to the results obtained by Cenkowski and Sosulski (1998) where an increase in starch swelling was observed.

When high amylose starch is exposed to a hydrothermal treatment, the amylose molecules in the granule can realign through hydrogen bonding involving other amylose, amylopectin and/or lipids (Hoover & Manuel, 1996a). These associations may result in physically induced crosslinked starch, which has reduced swelling potential during subsequent gelatinisation. The possible existence of such crosslinks involving starch in moisture-conditioned and micronised cowpeas in this research was studied through enzymatic digestion and HPLC-GP. Retrograded starch is resistant to α-amylase hydrolysis and is referred to as resistant starch type-III (Karim, Norziah, & Seow, 2000). Micronisation (41 % moisture, 130, 153 170 ºC) significantly reduced the level of digestible amylose (Table 3.3.1).

HPLC-GP analysis of the samples showed that micronisation of moisture-conditioned seeds to 170 °C did not have a significant ($P \leq 0.05$) effect on the solubility of amylopectin; however the treatment significantly ($P \leq 0.05$) reduced the amylose solubility from 11.6 to 8.8 % (Figure 3.3.8). The reduced solubility for amylose at 98 °C is possibly due to amylose-amylose associations formed during starch retrogradation. Amylose-amylose associations have a higher melting point (in the range of 120-170 °C) than amylose-amylopectin associations (40-100 °C) (Karim *et al*., 2000) and hence could not have been dispersed into solution at the temperature (98 °C) used in this study. Hoover and Manuel (1996a) reported similar decrease in amylose leaching following heat moisture treatment of legume starches.

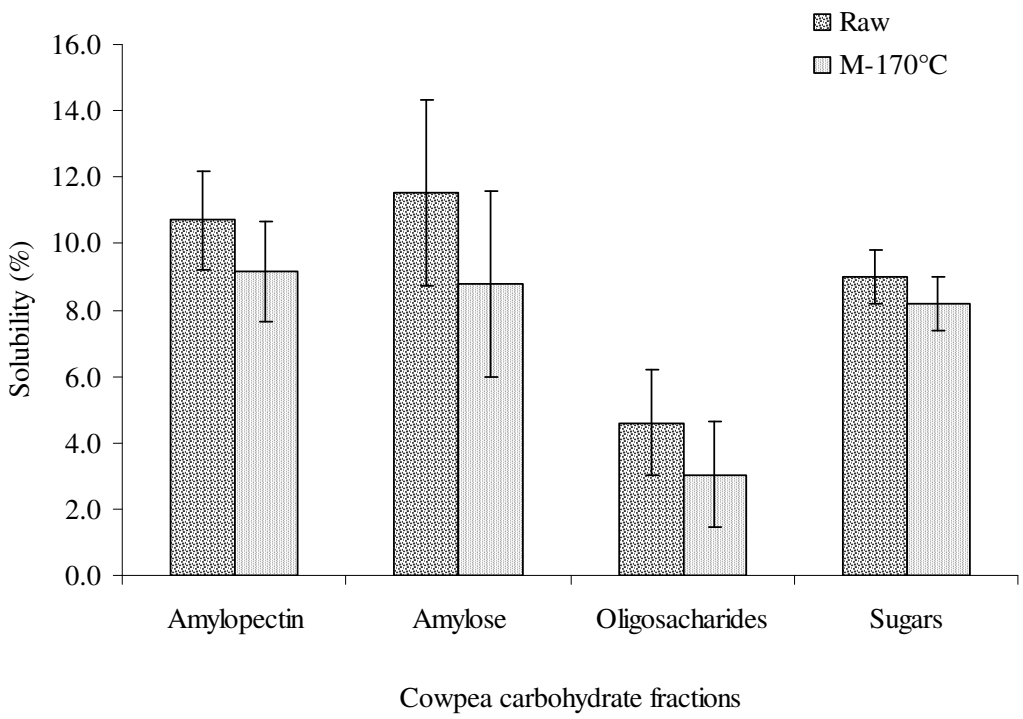

Figure 3.3.8 Effect of micronisation temperature of 170 °C on solubility of amylopectin, amylose, oligosaccharides and sugars from micronised (41 % moisture 170 °C) seeds, (vertical bars indicate standard deviations of the means M - 170 °C = Micronised (41 % moisture 170 °C))

The paste viscosity for the M-170 °C flour gradually increased throughout the heating, holding and cooling periods without forming a peak. The gradual increase in viscosity during the pasting process could be ascribed to the gelatinisation of residual starch granules (Figure 3.3.9), which comprised approximately 11 % of the total cowpea starch content.

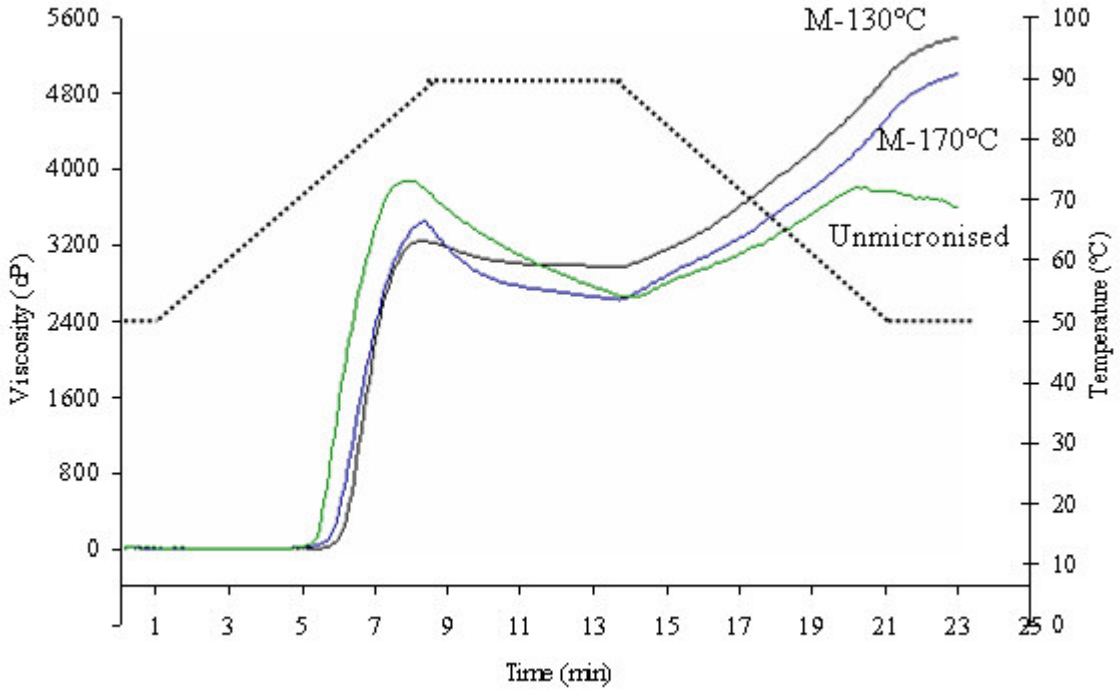

Figure 3.3.9 **Effect of micronisation temperature (130 °C and 170 °C) on pasting properties of cowpea starch isolated from micronised seeds, (Unmicronised= Raw, M= Micronised (41 % moisture, 130 and 170 °C))**

The gelatinised starch in M-170 °C was possibly also depolymerised. Depolymerised polysaccharides cannot reorder to the same extent as relatively intact polysaccharides molecules. Therefore, their contribution towards set back viscosity during the cooling phase would be negligible. In order to ascertain the possible presence of depolymerised starch in the M-170 °C flour, water solubility of starch and its degradation products were analysed. The HPLC-GP results (Figure 3.3.8) do not show extensively depolymerised starch due to micronisation (M-170 °C) i.e., no change in water-soluble sugars and oligosaccharides for this sample. Van den Einde *et al*. (2004) demonstrated that thermal (110, 140, 160 °C) treatment of low moisture (30 and 43 % moisture) corn starch in the absence of shear, resulted in endodegradation of starch and not exodegradation. Exodegradation of starch is indicated by increase in water-soluble products.

The presence of damaged starch, indicative of possible crosslinking and/or depolymerisation, was further elucidated by light microscopy. M-170 °C flour contained intact cells which absorbed acid Fuchsin (Figure 3.3.6 (1c)) and Congo red stains (Figure 3.3.10 (a) and 3.3.10 (b)) indicating the presence of protein and gelatinised starch, respectively.

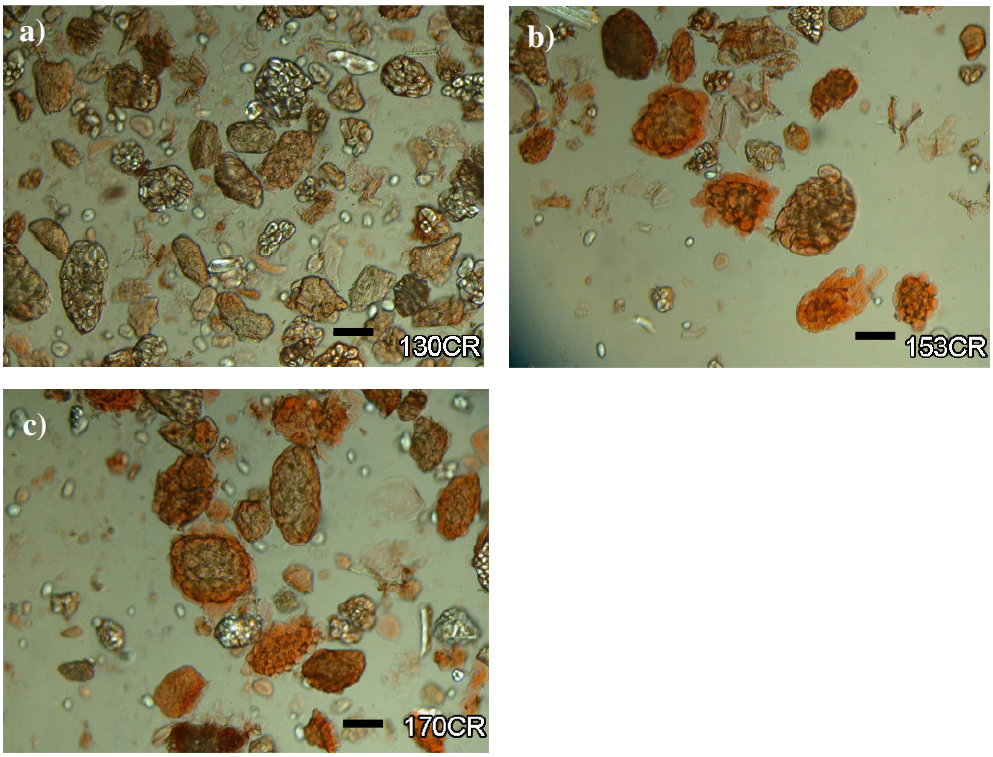

Figure 3.3.10 Effect of micronisation temperatures (130 °C, 153 °C and 170 °C) on cowpea flour from micronised (41 % moisture, infrared heating) seeds stained for damaged starch with Congo red: a = M-130 ºC, b = M-153 °C and c = M-170 ºC, Bar ~ 50 nm

When the cowpeas flour was digested with enzymes, the intact cells were observed to be partially susceptible to α-amylase (Figure 3.3.11 (2b) and 3.3.11 (2c)), and protease (Figure 3.3.11 (3b) and 3.3.11 (3c)) but were indigestible with pectinase ((Figure 3.3.11 (4c) .

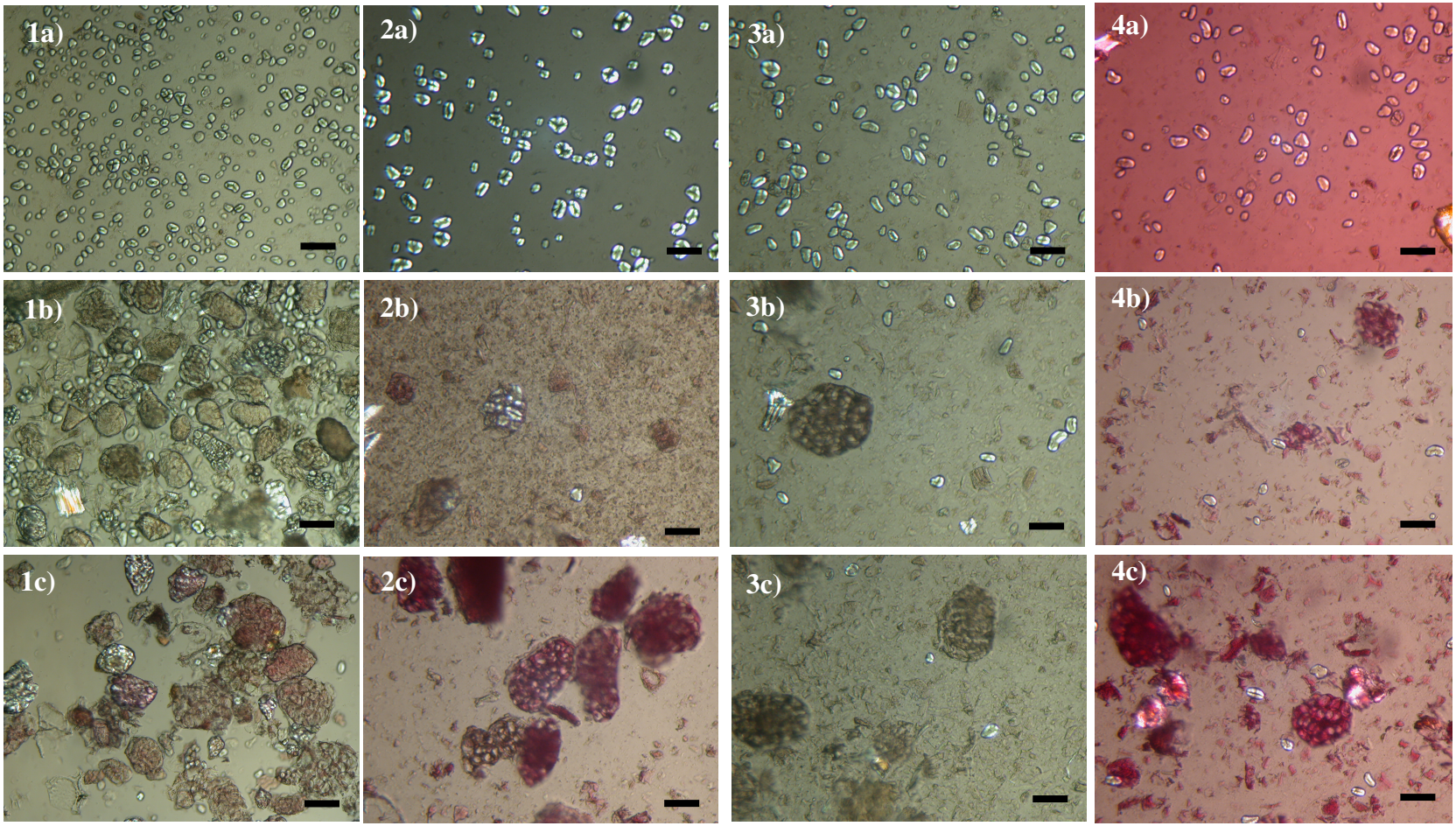

Figure 3.3.11 Effect of micronisation temperatures on cowpea flour from micronised (41 % moisture, 130 °C and 170°C) seeds during sequential enzyme digestion (stained with acid Fuchsin): 1 = No enzyme treatment, 2 = α –amylase, 3 = α –amylase - protease, and 4 = α –amylase-protease- pectin; a = unmicronised, b = M-130 ºC and c = M-170 ºC, Bar ~50 nm

3.3.4 Conclusions

Micronisation of moisture-conditioned cowpeas to temperatures up to 170 °C is beneficial in terms of reduced cooking time of whole seeds. Micronisation of moisture conditioned cowpeas to 130 °C is more effective in reducing the cooking time of cowpeas and would be recommended for this process. This will impact positively on energy and time requirements for cooking dry cowpeas. However, starch functionality is compromised by excessive micronisation as exhibited by M-170 °C cowpea samples.

3.4 Effect of micronisation temperature (130 °C and 170 °C) on functional properties of cowpea flour[4]

Abstract

Functional properties of cowpea flour from moisture-conditioned (41 %) seeds micronised to two different surface temperatures (130 °C and 170 °C) were studied. Micronisation (41 % moisture, 130 °C and 170 °C) significantly ($P \leq 0.05$) increased the water absorption capacity and least gelation concentration of the flour. The treatment significantly ($P \leq 0.05$) reduced the water solubility and swelling indices, gel strength and foaming capacity of the flour. The changes in cowpea flour functional properties, such as the loss of foaming capacity in flours from micronised (41 % moisture, 130 °C and 170 °C) seeds were associated with significant ($P \leq 0.05$) increase in surface hydrophobicity and cross linking of the cowpea protein. SDS-PAGE of the protein-rich fractions revealed changes in the protein subunit profile, which included the formation of disulphide bonds and possibly Maillard reaction derived cross links. The flour from M-170 °C seeds was significantly ($P \leq 0.05$) darker than the flour from unmicronised and M-130 °C seeds.

Keywords: cowpea flour, micronisation, protein, functional properties

[4] Submitted for publication in *Food Chemistry*

3.4.1 Introduction

Micronisation is a short-time, high-temperature process that utilises electromagnetic radiation in the infra red region (wavelength of 1.8-3.4 nm) to rapidly heat materials (Zheng *et al*., 1998). The process has been shown to considerably reduce the cooking time of legumes such as split peas, lentils and cowpeas, (Cenkowski & Sosulski, 1998; Arntfield *et al*., 2001; Phadi, 2004). However, wider use of the micronisation process in cowpeas could be attained by extending the utilisation of micronised legume seeds in the diet beyond the whole seed. Milling of moisture-conditioned and micronised cowpeas into flour could be one such process since there are existing uses of cowpea flour in food systems. There is a wide variety of products that are made from cowpea flour in different parts of Africa which are dependent on its functionality (Phillips *et al*., 2003; Hallén *et al*., 2004; McWatters *et al*., 2005). Apart from the traditional products, cowpea flour has also been used as a nutritious ingredient in fried (Kerr *et al*., 2001) and baked (Phillips *et al*., 2003) products as well as comminuted meat products such as chicken nuggets (Prinyawiwatkul *et al*., 1997b) and meatballs (Serdaroglu *et al*., 2005).

The suitability of cowpea flour in such food systems is dependent on its functional properties such as foaming, water and oil absorption capacities (WAC and OAC) as well as thermally induced gelling (Prinyawiwatkul *et al*., 1997a; Abu *et al*., 2005). Cowpea protein (24 %) is one of the main contributing components to the functionality of the flour. Cowpea seeds have high protein content which is relatively hydrophilic and water soluble (Mwasaru *et al*., 1999a). These physicochemical properties of cowpea protein are crucial in retaining the good foaming properties of the flour which are necessary in imparting a spongy texture of cowpea flour based products such as *akara* (Plahar *et al*., 2006). The WAC and OAC and gelling properties of heterogeneous systems such as flour are a factor of the physicochemical characteristics of protein and starch components (Prinyawiwatkul *et al*., 1997b).

Micronisation of moisture-conditioned seeds has been shown to precook legumes such as peas (Cenkowski & Sosulski, 1998), lentils (Arntfield *et al*., 2001), cowpeas (Phadi, 2004), and beans (Bellido *et al*., 2006). In addition to whole seed products, the flour from

moisture-conditioned and micronised cowpeas has the potential for utilisation in some food systems depending on its functionality. 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). Similarly, Cenkowski and Sosulski (1998) reported significant increase in pasting properties for flour milled from micronised (26 % moisture, 120 °C) split peas. Contrary to these findings, results reported in section 3.3.3.2 show that micronisation of moisture-conditioned (41 %) cowpea seeds, especially at 170 °C adversely reduced the pasting properties of cowpea flour. Fasina *et al*. (1999) reported improved water absorption capacity for hulless and pearled burley following micronisation (26.5 % moisture, 105 and 115 °C). At the same time, a reduction in WAC has been reported for chick pea flour milled from micronised (17 % moisture, 69, 88 and 90 °C) seeds (Sarantinos & Black, 1996). Micronisation (17 % moisture, 69, 88 and 90 °C) has been reported not to have a definite effect on oil absorption capacity of chick pea flour (Sarantinos & Black, 1996). Since information on functional properties of flour from moisture-conditioned and micronised legumes is rather scanty, the objective of this study was to examine the effect of micronising moisture-conditioned cowpea seeds to low (130 °C) and high (170 °C) final surface temperatures on functional properties of the resultant flour.

3.4.2 Material and methods

3.4.2.1 Raw materials

Bechuana white (cream colour) cowpeas supplied by Agricol (Potchefstroom, South Africa) were cleaned to remove chaff, shrivelled and broken seeds. The cleaned seeds were packed in polypropylene bags and stored at 4 °C until the time of use.

3.4.2.2 Hydrothermal process and cowpea flour preparation

The cowpeas were micronised according to the method described in section 3.3.2.2.

3.4.2.3 Colour values of the cowpea flour

Colour of the unmicronised and micronised (41 % moisture, 130 and 170 °C) cowpea flour was determined using a Chroma Meter CR-400 (Konica Minolta Sensing Inc,

Osaka, Japan). The colour of the flours was expressed as L*, a*, b* values, where L*=lightness, a* =redness, and b*=yellowness.

3.4.2.4 Determination of moisture content

Moisture content of the micronised (41 % moisture, 130 and 170 °C) and unmicronised flours was determined using the AACC method 925.10 (American Association of Cereal Chemistry, 2000).

3.4.2.5 Determination of crude protein

Crude protein content in the cowpeas was determined using the Dumas method as reported in section 3.1.2.2.

3.4.2.6 Determination of nitrogen solubility index

Nitrogen solubility index of the flour was determined according to the AACC method 46 - 23 (AACC, 2000). One gram flour samples were dispersed in 50 ml of 0.1M NaCl solution and stirred continuously for 1 hr with the pH maintained at 7. About 20 ml of the suspension was centrifuged (10 000 *g*, 15 min, 4 °C) and the supernatant filtered through a Whatman No 1 filter paper. The nitrogen content of the filtrate was determined using a Leco nitrogen analyser 528 (Leco Africa Pty, Kempton Park, South Africa). Protein content of the filtrate was calculated using 6.25 as the conversion factor. Nitrogen solubility index was expressed as the percentage of sample protein on dry basis.

3.4.2.7 Determination of water solubility index (WSI), water and oil absorption capacities (WAC, OAC)

Water and oil absorption capacity (WAC, OAC) of the flour from unmicronised and micronised (41 % moisture, 130 °C and 170 °C) cowpeas were determined according to the AACC method 56-20 (AACC, 2000) with slight modifications. A 2 g (M0) flour sample was dispersed in 40 ml deionised water or refined sunflower oil and vortexed for 10 min. The samples were centrifuged (1000 *g*, 15 min at 20 °C) and the supernatant decanted. The centrifuge tubes were then inverted for 5 min on a paper towel followed by weighing of the residue (M2). The residue from the water absorption samples was

dried in a hot air oven for 24 h at 50 °C and weighed (M1). WAC, OAC and WSI were calculated as follows:

WAC or OAC = {(M2 –M0)/M0} x 100; and WSI = {(M0-M1)/MO} x 100

Where M0 = Sample weight (db); M1 = Weight of dried residue; M2 = Weight of wet or oily residue.

3.4.2.8 Determination of swelling index

Samples of flour from unmicronised and micronised (41 % moisture, 130 °C and 170 °C) cowpeas were dispersed in deionised water (1:20, w/v) and vortexed for 1 min followed by heating in a water bath at 90 °C for 30 min with intermittent mixing. The heated samples were cooled for 30 s under running water and for 10 min in an ice bath to accelerate gel formation. The tubes containing the gels were centrifuged (4500 *x g*, 20 °C) for 10 min, after which the samples were allowed to stand for 5 min at 24 °C. The supernatant was decanted and the residue weighed. Swelling index was calculated as the ratio of the weight of the final residue to the initial sample weight (Abu *et al*., 2005).

3.4.2.9 Determination of cowpea flour concentration on gelation

Cowpea flour dispersions in deionised water with concentration from 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 % (w/v) were prepared in test tubes. The dispersions were heated in a water bath at 80 °C for 1 h followed by rapid cooling under running cold water. The test tubes were left to set at 4 °C for 2 h. Least gelation concentration was determined as the concentration when sample from the inverted tube did not fall or slip (Adebowale, Olu-Owolabi, Olawumi & Lawal, 2005).

3.4.2.10 Determination of gel strength

The strength of gels formed by the flour from unmicronised and micronised (41 % moisture, 130 °C and 170 °C) cowpeas was determined following the method reported by Abu *et al*. (2005). Eighteen percent (w/v) flour dispersions were heated in a water bath (90 °C) for 50 min. The hot pastes were cooled in running water and allowed to set for 15 min in an ice bath following which the gels with a height of 20 mm were kept at 4 °C for 16 h. Gel strength was measured using a TA-XT2 texture analyser (Stable micro

systems, Goldalming, Surrey, UK). A 20 mm diameter probe (P20) with a punch area of 314.16 mm^2 was used. The probe operated at a pre-test speed of 1 mm s^{-1} and penetrated the gel at the speed of 0.5mm s^{-1}. Force required for the probe to penetrate the gel to 8 mm was recorded as a measure of gel strength.

3.4.2.11 Determination of foaming capacity of cowpea flour

The foaming capacity of the flour from unmicronised and micronised (41 % moisture, 130 °C and 170 °C) cowpeas was determined according to the method reported by Akubor, Isolokwu, Ugbane & Onimawo (2000). A 5 % (w/v) dispersion of the flour in deionised water was whipped using a Power Five mixer (Kenwood Ltd, Hants, England) for 8 min at 24 °C. The foam was quantitatively transferred into a graduated cylinder. Foam volume was expressed as a percentage of the volume occupied by the sample prior to whipping.

3.4.2.12 Extraction of a protein-rich fraction

Cowpea protein was extracted from unmicronised and micronised (41 % moisture, 130 °C and 170 °C) seeds according to a modified method of Mwasaru *et al*. (1999a) and Horax *et al*. (2004a). The flour was defatted with hexane (Flour: Hexane, 1:6, w/v) at 25 °C for 4 h and air dried in a fume hood overnight. Ten percent of the defatted flour in 0.1 M NaOH at pH 8.5 was homogenised using a Ultra Turrax T25 (Janke and Kunkel GmbH & Co., K.G., Stauffen, Germany) at 24000 rpm for 30 min (4 °C) followed by centrifugation at 10 000 *g* (4 °C) for 1 h. The residues were extracted twice in 0.1 M NaOH at pH 8.5. The supernatants were pooled and precipitated by adjusting the pH to 4.5 using 0.1 M HCl. The precipitated protein was recovered by centrifuging followed by three washings with deionised water (pH 4.5). The protein isolate was solubilised by adjusting the pH to 7.0 followed by 24 h dialysis (pore size of 12-14 kDa, Labretoria, Pretoria, South Africa) at 4 °C. The dialysed material was freeze dried and termed protein-rich fraction (PRF). The nitrogen content of the PRF was determined using a Leco Nitrogen Analyzer 528 (Leco Africa Pty, Kempton Park, South Africa). Protein content of the PRF was calculated using 6.25 as the conversion factor.

3.4.2.13 Determination of protein surface hydrophobicity

Surface hydrophobicity of the PRF samples was determined according to the method of Hayakawa and Nakai (1985). Ten millilitre solutions of protein were made in 0.01 M phosphate buffer (pH 7) with concentrations ranging from 0.0001 to 0.0008 % (w/v). The probe for aromatic hydrophobicity, 1-anilino-8- naphthalene sulphonate (ANS) (25 µl, 8 mM in 0.01 M phosphate buffer (pH 7.0)) was added into each protein solution, and fluorescence intensities of these solutions were measured at 390 nm excitation and 470 nm emission using an LS 30 Luminescence spectrometer (PerkinElmer Inc, Boston, MA). The surface hydrophobicities, expressed as a slope of fluorescence intensity (arbitrary units) against protein concentration were calculated by linear regression (Statistica 6.0).

3.4.2.14 Determination of dityrosine

Formation of dityrosine in moisture-conditioned and micronised samples was determined according to the method of Davies, Delsignore & Lin (1987). Protein-rich fraction was dissolved in 0.1 M HEPES buffer (pH 7.0) to make a 0.53 mg protein in 2 ml solution. Dityrosine fluorescence was measured at 325 nm excitation and 410 – 420 nm emission using an LS 30 Luminescence spectrometer (PerkinElmer Inc, Boston, MA).

3.4.2.15 Gradient SDS –PAGE of the protein-rich fraction (PRF)

The effect of micronisation temperature on the molecular size and distribution of cowpea proteins was studied using gradient SDS-Gel electrophoresis according to the method reported by Byaruhanga, Erasmus and Taylor (2005). The gels (12.5 x 16 cm, 1.5 mm thick) had a concentration gradient from 4 to 18 % and were polymerised with 0.1 % (w/v) ammonium persulphate (APS) and tetramethylenediamine (TEMED). The protein was dispersed in sample buffer (0.125 % (w/v) Tris/HCl, 20 % (v/v) glycerol, 2 % (w/v) SDS and 0.005 % (w/v) bromophenol blue). For the electrophoresis under reducing conditions, 0.1 % 2-mercaptoethanol was added to the sample buffer. The gels were loaded to a constant protein content of 49.5 µg. Molecular weight marker solution, low range (Roche Diagnostics Corporation, Indianapolis, USA) was diluted by 1 to 10 with reducing sample buffer. The mixture consisted of phosphorylase B (M_r 97.4 x 10^3),

bovine serum albumin (M_r 66.2 x 10^3), aldolase (M_r 39.2 x 10^3), triose phosphate isomerase (M_r 26.6 x 10^3), trypsin inhibitor (M_r 21.5 x 10^3) and lysozyme (M_r 14.4 x 10^3). The diluted molecular weight marker was boiled for 5 min and 20 µl (96 µg protein molecular weight marker) was loaded on the gels. Electrophoresis was carried out at a constant voltage of 25 mA per gel and 150 V for 14 h at 8 °C using a Protean II xi vertical cell with a 1000 Powerpac (Bio-Rad Laboratories, Hercules, CA). Proteins were stained with 0.03 % (w/v) Coomassie Brilliant Blue R250 in 7 % (v/v) acetic acid and 20 % (v/v) methanol and 3.2 % trichloroacetic acid (TCA). After staining the gels were de-stained with 4 % (v/v) acetic acid and 29 % (v/v) methanol and 3 % TCA. The de-stained gels were scanned on a flat bed scanner.

3.4.2.16 Statistical analysis

Mean values for the functional properties, colour and surface hydrophobicity of the cowpea flours were obtained from three repetitions. One way analysis of variance (ANOVA) of the data and correlations of variables was performed using Statistica version 6 (StatSoft, Inc., Tulsa, OK) statistical software. The least significance difference test at $P \leq 0.05$ was used to separate the means.

3.4.3 Results and discussion

Cowpea flour from unmicronised seeds contained 23.9 % crude protein which is within the range reported for cowpeas (Chan & Phillips, 1994). Although there were no prominent changes in crude protein content with micronisation (41 % moisture, 130 and 170 °C) (Table 3.4.1), the amount of protein extracted as well as the purity of the PRF declined with increasing micronisation temperature (Figure 3.4.1). The protein recovery attained in this work is higher than what some workers have obtained for cowpea, although the protein content of the PRF is within the range reported for cowpea protein isolates (Mwasaru *et al*., 1999a)

Micronisation (41 % moisture, 130 °C and 170 °C) significantly ($P \leq 0.05$) reduced the lightness of cowpea flour with increasing temperature (Table 3.4.1). Cowpea flour from the M-170 °C seeds was the darkest of the samples and it had higher yellowness and

redness values. The browning of the cowpea flour with micronisation was possibly due to Maillard reactions since cowpeas do contain reducing sugars (Longe, 1980; Phillips *et al*., 2003) and have high protein content.

Table 3.4.1 Effect of high (170 °C) and low (130 °C) final micronisation temperature for cowpea seeds (41 % moisture) on physicochemical properties of cowpea flour

Physicochemical property	Micronisation		
	[y]Unmicronised	130 °C	170 °C
Moisture	9.2[a] (0.7)	5.0[b] (0.9)	4.8[b] (0.5)
Protein	24.2[c] (0.2)	24.7[b] (0.3)	25.9[a] (0.6)
L*	84.36[a] (0.4)	81.34[b] (0.6)	70.06[c] (0.3)
a*	5.00[c] (0.0)	5.23[b] (0.16)	8.45[a] (0.3)
b*	6.01[b] (0.2)	6.28[b] (0.4)	13.33[a] (0.3)
Protein solubility (%)	87.4[a] (13.7)	59.7[b] (13.0)	47.7[c] (5.7)
PRF surface hydrophobicity	228[c] (45)	369[b] (34)	624[a] (23)

Means followed by the same letter within a row are not significantly different at level $P \leq 0.05$; standard deviations of the means are in parenthesis, L*= lightness, a* = redness, b*= yellowness, PRF = Protein-rich fraction, [y] Unmicronised = Raw

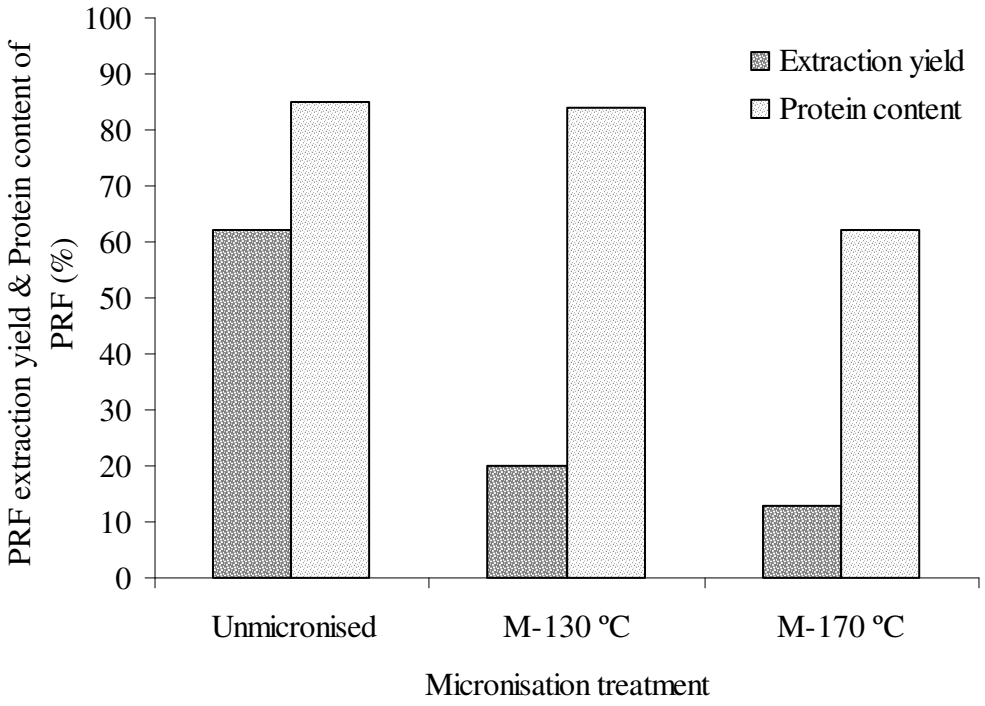

Figure 3.4.1 Effect of high (170 °C) and low (130 °C) final micronisation temperature for cowpea seeds (41 % moisture) on recovery of protein rich fraction (PRF) from cowpea seeds and its protein content (purity); (Yield has been expressed as percentage of crude protein content of the cowpeas)

Protein solubility in an aqueous environment is an essential property that affects functionality of cowpea flour in terms of foaming, emulsification and gelation (Nnanna, Phillips, McWatters & Hung, 1990). Nitrogen solubility index of unmicronised flour was comparable to values reported in literature (Abu *et al.*, 2005) (Table 3.4.1). Micronisation (41 % moisture, 130 °C and 170 °C) reduced ($P \leq 0.05$) the NSI of the cowpea flour by 32 and 45 %, for the M-130 °C and M-170 °C flours, respectively.

Micronisation (41 % moisture, 130 °C and 170 °C) caused the denaturation of the cowpea protein hence reducing its solubility. Similar reductions in protein solubility have been reported in micronised peas (Cenkowski & Sosulski, 1998), lentils (Arntfield *et al.*, 1997;

Arntfield *et al*., 2001) and beans (Bellido *et al*., 2006). Zheng *et al*. (1998) attributed the reduction in protein solubility for micronised (18 % moisture, 140 °C) legumes to hydrophobic interactions which render the protein less soluble in water. Cowpea protein is generally hydrophilic (Horax *et al*., 2004a) indicating that most of the non polar/hydrophobic side chains are buried inside the protein. The protein-rich fraction from unmicronised cowpeas had lower surface hydrophobicity values as compared to what is reported for cowpea protein isolate (Table 3.4.1) (Horax *et al*., 2004a). The difference in surface hydrophobicity of the cowpea proteins could be due to difference in variety (Horax *et al*., 2004a) and protein isolation methods. However, micronisation (41 % moisture, 130 °C and 170 °C) significantly ($P \leq 0.05$) increased the surface hydrophobicity of the PRF possibly by changing the cowpea protein conformation to expose more hydrophobic sites. The PRF from the M-170 °C had significantly higher surface hydrophobicity than the PRF from M-130 °C cowpeas (Table 3.4.1). This may imply that more hydrophobic sites were exposed with increasing micronisation temperature.

Furthermore, possible complexion of phenolic compounds and tannins with protein could contribute to the reduction in protein solubility of the micronised (41 % moisture, 130 °C and 170 °C) cowpeas (Chang, Collins, Bailey & Coffey 1994). Phenolic compounds and tannins have been reported in cowpea varieties (Chang *et al*., 1994; Cai, Hettiarachchy & Jalaluddin, 2003). Phenolic compounds are oxidised to quinones that react irreversibly with sulphydryl and amino groups of proteins hence reducing protein solubility (Damodaran, 1996a).

Cowpea flour from unmicronised seeds absorbed 1137 g kg^{-1} oil and micronisation (41 % moisture, 130 °C and 170 °C) did not change this attribute (Table 3.4.2). Prinyawiwatkul *et al*. (1997a) reported that oil absorption capacity of cowpea flour does not change with most processing treatments such as boiling, milling particle size and fermentation. The presence of non-polar side chains, which bind the hydrocarbon side chain of oil would promote oil binding capacity of flours, however in this study the significantly ($P \leq 0.05$) higher surface hydrophobicity (Table 3.4.1) of the protein-rich fraction isolated from

cowpeas (41 % moisture) micronised to 170 °C did not enhance the OAC of the flour. Oil absorption capacity is an important property for cowpea flour that would be used as an extender in comminuted meat formulations such as meat balls and sausages where flavour retention and palatable mouth feel is desired. Since micronisation (41 % moisture, 130 °C and 170 °C) did not change the OAC of the flour, flour from moisture-conditioned and micronised seeds could be used to extend meat products without adverse effect on texture and mouth feel (Prinyawiwatkul *et al*., 1997b; Serdaroglu *et al*., 2005).

Table 3.4.2 Effect of high (170 °C) and low (130 °C) final micronisation temperature for cowpea seeds (41 % moisture) on functional properties of cowpea flour

Functional property	Micronisation		
	[y]Unmicronised	130 °C	170 °C
Oil absorption capacity (g kg^{-1})	1137 (174)	1208 (177)	1177 (147)
Water absorption capacity (g kg^{-1})	1384[c] (89)	2509[b] (75)	2871 [a] (101)
Water solubility index	38.6[a] (1.6)	22.6[b] (1.51)	17.4 [c] (1.6)
Swelling index	7.24[a] (0.43)	5.95[b] (0.44)	5.92 [b] (0.39)
Gel strength (Nmm^2)	115.7[a] (8.9)	72.3[b] (4.6)	28.8 [c] (2.5)
Least gelation capacity (w/v)	8[c] (0.8)	11[b] (1.0)	13[a] (1.0)
Foam capacity (%)	291[a] (11.5)	112[b] (2.1)	102 [c] (2.1)

Means followed by the same letter within a row are not significantly different at level $P \leq 0.05$; standard deviations of the means are in parenthesis [y] = Raw

Micronisation of moisture-conditioned cowpea seeds (especially 170 °C) significantly ($P \leq 0.05$) increased the WAC of the cowpea flour (Table 3.4.2). The flour from M-170 °C cowpeas absorbed more ($P \leq 0.05$) water than M-130 °C samples. Fasina *et al*. (1999) reported that micronisation (26 % moisture, and 115 °C) improved the water holding capacity of hulless and pearled barley flour. Prinyawiwatkul *et al*. (1997a) also

reported improved water retention capacity for cowpea flour from soaked and boiled seeds. Starch and protein are important constituents that determine water absorption properties of heterogeneous systems such as flour. Fasina *et al*. (2001) demonstrated that micronisation (<10 % moisture, 140 °C) of legumes (pinto and black beans, lentils and green peas) resulted in increased WAC which was mainly attributed to protein denaturation since the amount of starch gelatinised under the micronisation conditions was minimal.

Despite the increase in water absorption capacity with micronisation (41 % moisture, 130 °C and 170 °C), swelling index of the flour had a significant ($P \leq 0.05$) negative correlation ($r = -0.83$) with WAC. Micronisation (41 % moisture, 130 °C and 170 °C) significantly ($P \leq 0.05$) reduced the swelling index of the flours by 17.8 and 18.2 % for the M-130 °C and M-170 °C, respectively (Table 3.4.2). Swelling index is indicative of starch granule swelling during gelatinisation as well as water retention due to protein gelation. It has been reported that the starch in moisture-conditioned and micronised legumes is gelatinised and protein solubility is reduced (Arntfield *et al*., 1997; Bellido *et al*., 2006). Hence the flour from micronised (41 % moisture, 130 °C and 170 °C) cowpeas had lower swelling indices than the unmicronised cowpea flour. Similar reduction in swelling index has been reported in irradiated cowpea flour (Abu *et al*., 2005).

Cowpea flour from unmicronised seeds had a water solubility index (WSI) of 38.67 which was significantly ($P \leq 0.05$) reduced by 42 and 55 % by micronisation to 130 °C and 170 °C, respectively (Table 3.4.2). Water solubility index is an indication of the water soluble fractions in the flour such as protein and sugars. WSI had a significant positive correlation with NSI of the flour. Hence the reduced WSI could partly be due to the reduced protein solubility.

Cowpea flour from unmicronised cowpeas had the lowest ($P \leq 0.05$) gelation concentration of 8 % and formed a significantly ($P \leq 0.05$) stronger gel at 18 % concentration than the flour samples from moisture-conditioned seeds micronised to

130 °C and 170 °C cowpeas (Table 3.4.2). The least gelation concentration found in this work is within the values reported for cowpea flour (Olaofe *et al*., 1993; Prinyawiwatkul *et al*., 1997a). Micronisation (41 % moisture, 130 °C and 170 °C) increased the lowest gelation concentration to 10 and 12 % for the flour from moisture-conditioned cowpeas micronised to 130 °C and 170 °C, respectively. The strength of the gels formed from the 18 % (w/v) dispersion was significantly ($P \leq 0.05$) reduced by 39 and 75 % for the M-130 °C and M-170 °C flours, (Table 3.4.2). Prinyawiwatkul *et al*. (1997a) reported similar increase in the least gelation concentration for cowpea flour following soaking and boiling. In a heterogeneous system where both the protein and starch are in their native form, both protein and starch contribute towards the gelation properties of the flour. As indicated in section 3.3.3.2, micronisation of moisture-conditioned cowpea seeds to 170 °C severely reduced the pasting viscosities of the milled flour than the mild 130 °C micronisation treatment. Consequently the significant ($P \leq 0.05$) increase in the least gelation concentration and the weak gel formed by the cowpea flour from seeds micronised to 170 °C was most likely due more to protein gelation than starch gelation. In addition, Prinyawiwatkul *et al*. (1997a) reported that high protein (denatured) flour with pregelatinised starch require greater flour concentration for thermal gel formation to occur.

Foaming reflects the capacity of protein to form stable layers surrounding gas droplets in a liquid phase. In order for this to be possible, the proteins need 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). Cowpea flour from unmicronised seeds formed 191 ml of foam as compared to the 12 and 2 ml formed by M-130 °C and M-170 °C flour from the moisture-conditioned cowpeas micronised to 130 and 170 °C, respectively (Table 3.4.2). Similar reduction in foaming capacity has been reported in irradiated cowpea flour (Abu *et al*., 2005). The significant ($P \leq 0.05$) reduction in foaming capacity of micronised (41 % moisture, 130 °C and 170 °C) samples was possibly due to extensive protein denaturation. Foaming capacity had a significant ($P \leq 0.05$) positive correlation ($r = 0.79$)

with protein solubility index of the flour. Aluko and Yada (1993) reported that protein solubility has a positive correlation with foaming capacity/expansion while aliphatic hydrophobicity has a negative impact. In addition, Townsend and Nakai (1983) reported that protein flexibility is a crucial property for protein stabilised foams. Reduction in foaming properties of partially purified cowpea globulin treated with microbial calcium-independent transglutaminase has shown that protein crosslinking may lead to gradual loss of flexibility and the proteins' ability to unfold at the water air interface (Aluko & Yada, 1999).

SDS-PAGE of the extracted protein-rich fraction from the unmicronised cowpeas showed two main (56 and 50 kDa) and two minor (63 and 61 kDa) bands in the region between 39.2 and 66 kDa and 5 minor bands in the 20 to 39 kDa regions (Figure 3.4.2 (a)). These bands correspond to the four major polypeptides (65, 60, 56 and 50 kDa) reported by Chan and Phillips (1994) in the globulin fraction. The cowpea albumins have bands in the 90 - 100 kDa regions and minor bands were observed in this region (Figure 3.4.2 (a)). Rangel *et al*. (2003) observed two major bands 50 and 52 kDa following SDS-PAGE of cowpea protein isolate. These are in the typical molecular mass range for 7S storage proteins (Horax *et al*., 2004a).

Although disulphide links have been reported in the α- vignin component of purified cowpea globulins involving a 60 and a 20 subunits, there was no distinct changes in the subunit profile of the PRF from unmicronised cowpea seeds under reducing conditions (Figure 3.4.2 (b)). There was relative reduction in the size of the 2 major bands in the protein isolated from moisture-conditioned cowpea seeds micronised to 170 °C (Figure 3.4.2(a)). The reduction in size could possibly be due to associations involving the 52 and 55 kDa polypeptides in the formation of higher molecular weight polymers that could not pass through the gradient gels.

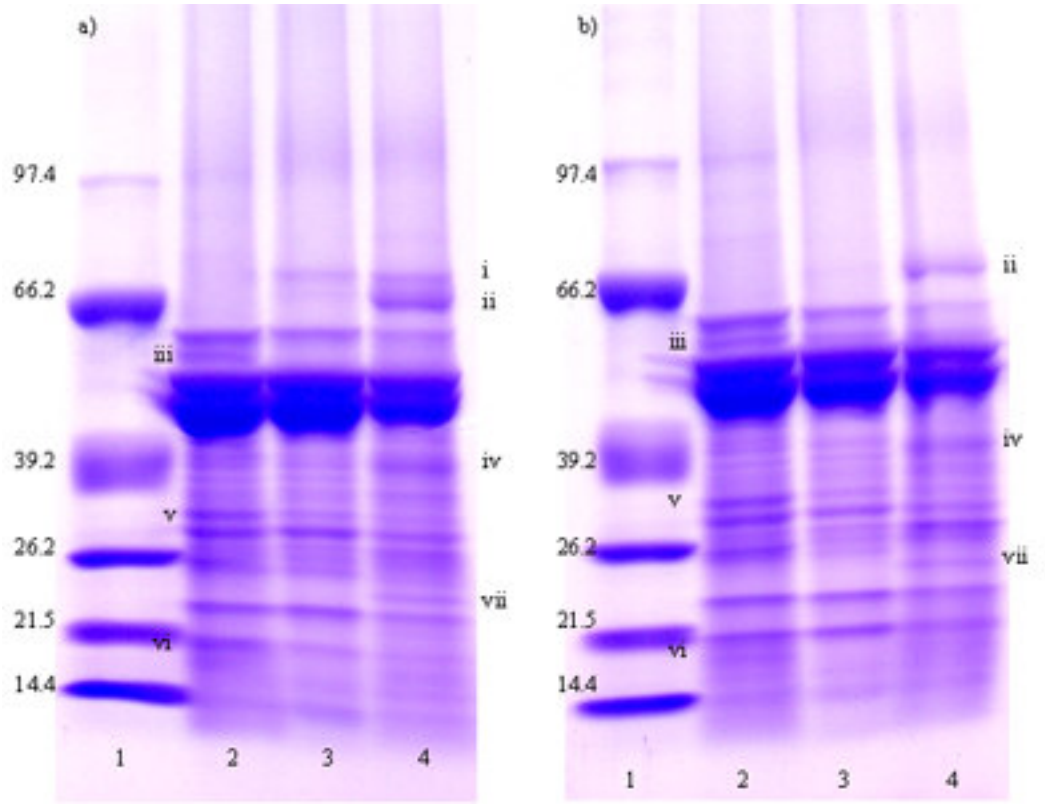

Figure 3.4.2 SDS-gradient gel electrophoresis profiles of cowpea protein-rich fraction from unmicronised (raw) and micronised (41 % moisture, 130 and 170 °C) seeds under non reducing (a) and reducing conditions (b): lane 1 = molecular markers; lane 2 = unmicronised; lane 3 = micronised to 130 °C and lane 4 = micronised to 170 °C (changes in protein band profile are denoted with (i) to (vi))

Micronisation (41 % moisture, 130 °C and 170 °C) also resulted in the formation of 2 minor bands, at 69 and 73 kDa (Figure 3.4.2(a – i) and 3.4.2 (a-ii)), and the 69 kDa (Figure 3.4.2(a –ii)) band was prominent in the fraction extracted from moisture-conditioned cowpeas micronised to 170 °C. The 73 kDa (Figure 3.4.2(a-i)) was possibly a result of disulphide cross-links involving the 21 kDa and 61 kDa monomers, since it was reduced by mercaptoethanol (MCE) (Fig 2 (b)). The 61 (Figure 3.4.2(a) and 3.4.2 (b-iii)) and 21 kDa monomers (Figure 3.4.2(a) and 3.4.2(b-vi)) disappeared in micronised

(41 % moisture, 130 °C and 170 °C) samples under non-reducing conditions and reappeared under reducing conditions (Figure 3.4.2(a) and 3.4.2(b)). Freitas *et al*. (2004) reported that the γ – Vignin (22 kDa) fraction of cowpea globulins have an intra-polypeptide disulphide bond. It is possible that micronisation of moisture conditioned seeds especially to 170 °C resulted in the reformation of these bonds leading to the formation of interpolypeptide disulphide cross linking.

In addition to disulphide cross linking, it is evident that other forms of cross linking may have taken place during micronisation (41 % moisture, 130 °C and 170 °C). Other bands that were present (Figure 3.4.2(a); 3.4.2(b – ii); 3.4.2(b-iv); 3.4.2(b- vii)) in the protein-rich fraction from moisture-conditioned cowpeas micronised to 170 °C were not reduced by MCE and could possibly be due to other forms of cross-linking that involved the 2 prominent bands and band (v) (Figure 3.4.2 (b-v)), since these bands decreased with micronisation temperatures both under reducing and non reducing conditions (Figure 3.4.2(a) and 3.4.2(b)). Maillard reactions might have led to the formation of some crosslinks in the cowpeas protein (Gerrard, 2002). The decrease in L* values with increasing micronisation temperatures indicate that Maillard-type browning reactions possibly occurred in micronised (especially the 170 °C) sample. The higher end micronisation temperature (170 °C) resulted in darker flour than the lower micronisation temperature (130 °C) (Table 1). Cowpeas contain both reducing and non reducing sugars (Longe, 1980; Phillips *et al*., 2003) which could result in both Maillard and caramelisation reactions. In addition, dityrosyl and isopeptide cross links would also be another possible form of crosslinking (Singh, 1991; Gerrard, 2002) due to the severe heat treatment especially for the flour from the moisture-conditioned cowpeas micronised to 170 °C. Measurement of dityrosine formation was not successful due to the presence of impurities in the extracted protein-rich fractions.

3.4.4 Conclusions

Micronisation of moisture-conditioned cowpeas severely affects the functionality of cowpea protein resulting in the loss of foaming capacity and reduction in gelation capacity, solubility index and swelling. Despite this reduction, mild micronisation

temperatures can still be used to process flour with modified functionality. However, flours from moisture-conditioned cowpeas micronised to high temperatures have limited application in food systems due to the decline in most of the functional properties measured in this study.

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.

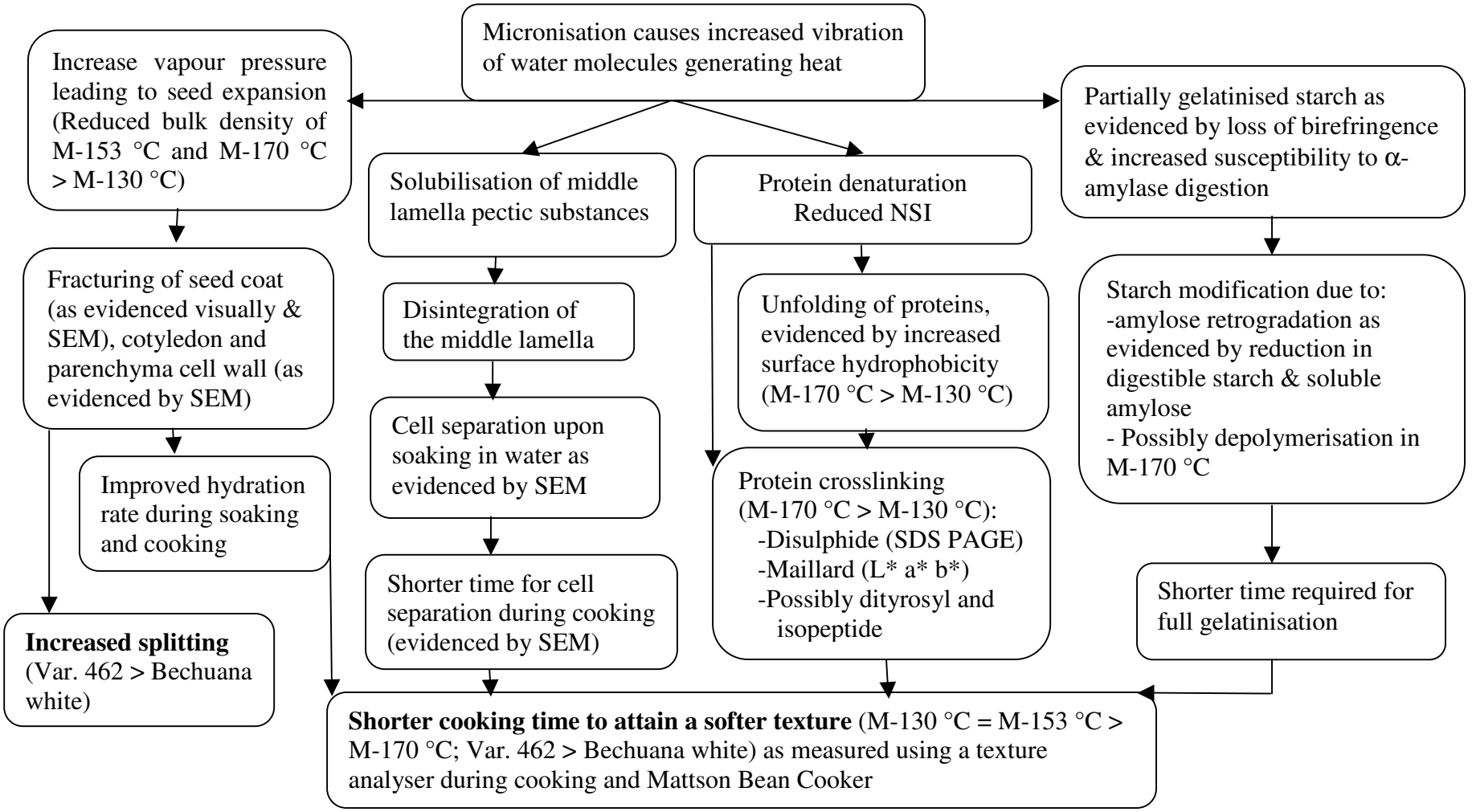

Figure 4.1 **Proposed mechanisms of the effect of micronisation (41 % moisture, 130, 153 and 170 °C) on cowpea seed structure and the physicochemical properties as related to cooking characteristics of cowpea seeds**

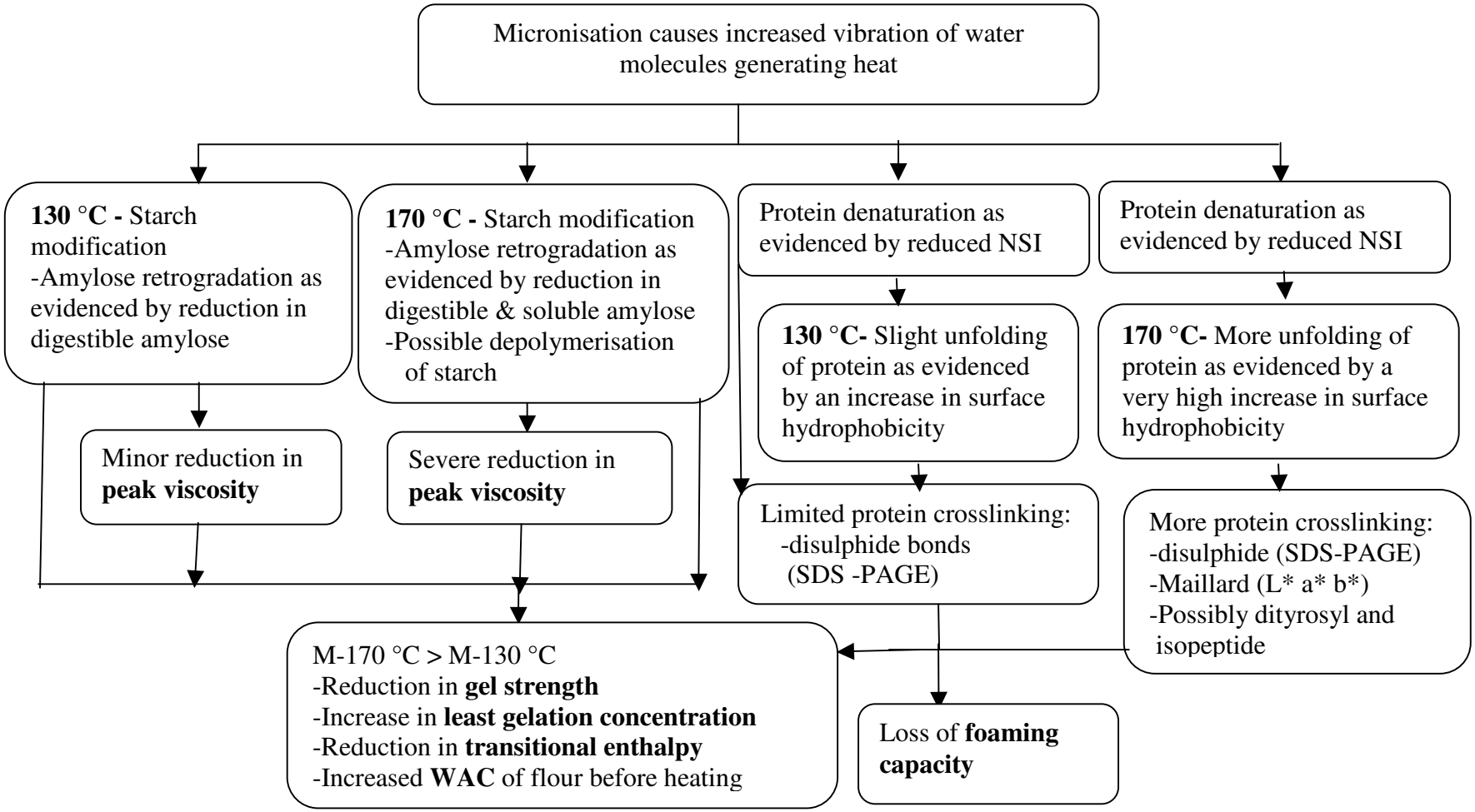

Figure 4.2 **Postulated effect of cowpea seed micronisation (41 % moisture, 130 and 170 °C) on physicochemical properties of starch and protein and functional properties of flour milled from the micronised (41 % moisture, 130 and 170 °C) seeds', NSI= Nitrogen solubility index, WAC= Water absorption capacity**

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.

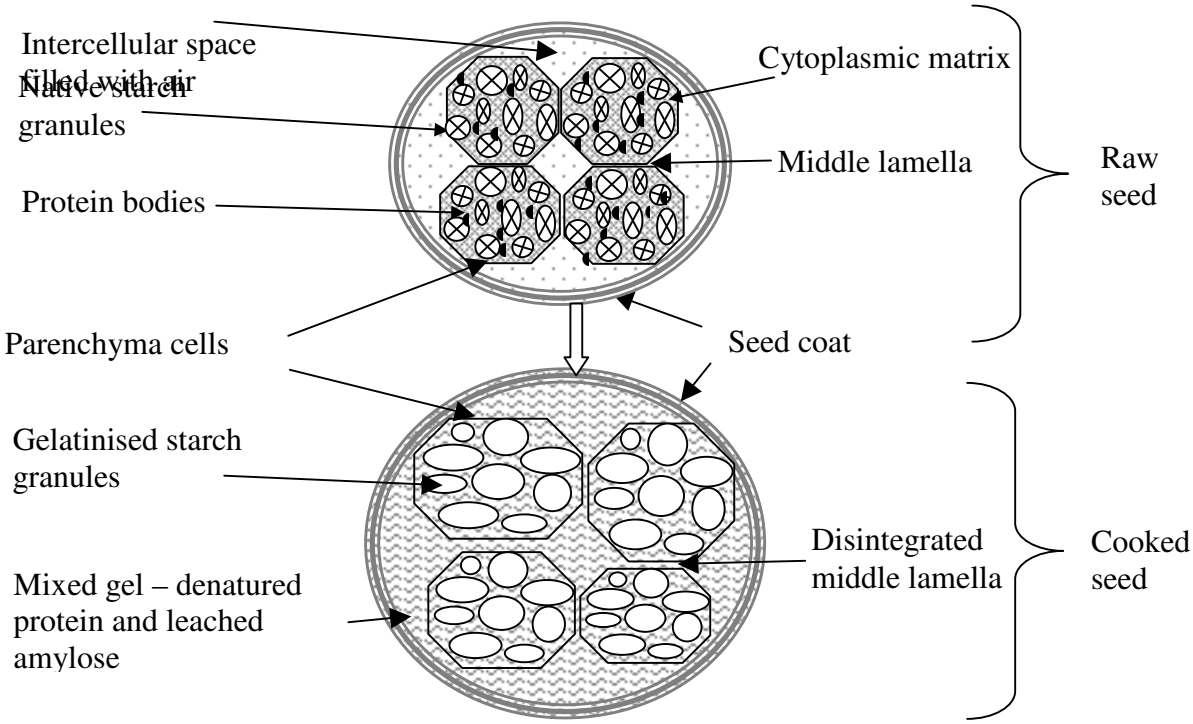

Figure 4.3 **Diagram showing the suggested changes in cowpea structure at the microscopical level that lead to softening of texture during cooking of unmicronised (raw) cowpeas** (Based on the mechanism proposed by Liu *et al*., 1992; Liu *et al*., 1993a; and Liu *et al*., 1993b)

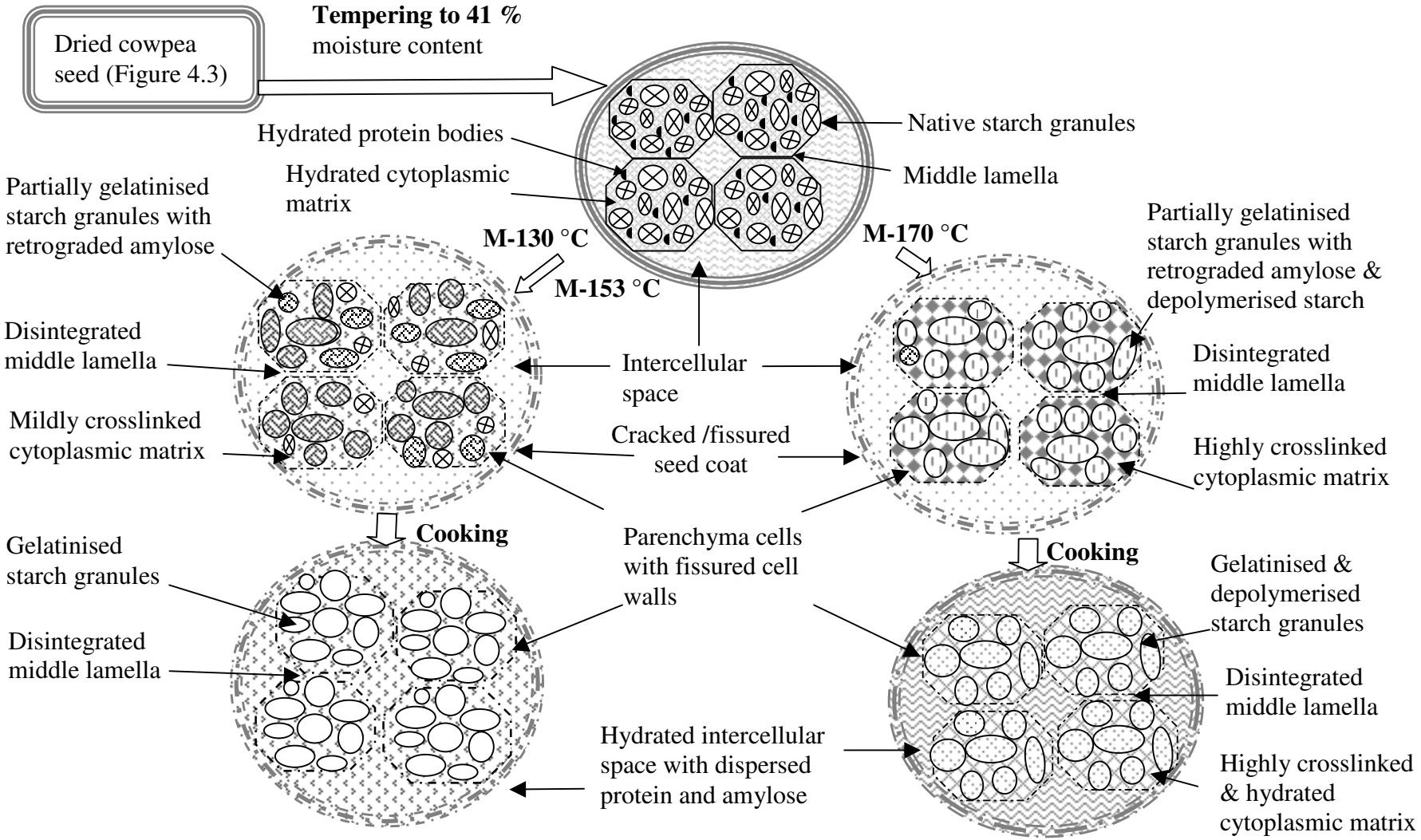

Figure 4.4 **Diagram showing the suggested changes in cowpea structure that lead to softening of texture during cooking of micronised (41 % moisture, 130, 153 and 170 °C) cowpeas**

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.

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PUBLICATIONS AND PRESENTATIONS

Peer reviewed publications

MWANGWELA, A.M., WANISKA, R.D. & MINNAAR, A. 2006**.** Hydrothermal treatments of two cowpea (*Vigna unguiculata* L. Walp) varieties: Effect of micronisation on physico-chemical and structural characteristics. *Journal of the Science of Food and Agriculture* 86, 35-45.

MWANGWELA, A.M., WANISKA, R.D., McDONOUGH C. & MINNAAR, A. 200X. Cowpeas cooking characteristics as affected by micronisation temperature: a study of the physicochemical and functional properties of starch. Accepted for publication in the *Journal of the Science of Food and Agriculture.*

MWANGWELA, A.M., WANISKA, R.D. & MINNAAR, A. 200X. Effect of micronisation temperature (130°C and 170°C) on functional properties of cowpea flour. Submitted to *Food Chemistry.*

Poster presentations

MWANGWELA, A.M., WANISKA, R.D., HALL, A. & MINNAAR, A. 2005. Effect of micronization temperature on structure and cooking characteristics of cowpea (*Vigna unguiculata* L. Walp) seeds. Poster presented at 1st International Edible Legume Conference and 4th World Cowpea Congress. Durban, South Africa. 17-21 April.

MWANGWELA, A.M., WANISKA, R.D., HALL, A. & MINNAAR, A. 2004. Structural changes during hydrothermal processing of cowpea seeds. Poster presented at the 43rd annual conference of the Microscopy Society of Southern Africa, Pretoria, South Africa, December.

ABU, J.O., BYARUHANGA, Y., EZEOUGO, L; FOMBANG, E. MWANGWELA, A.M. 2004. What's cooking with the electromagnetic spectrum? Poster presented at the South African Association of Food Science and Technology (SAAFoST) Student's

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Conference papers and proceedings

MWANGWELA, A.M. & MINNAAR, A. 2006. East and Southern Africa regional planning meeting for the Bean/Cowpea CRSP, Maputo, Mozambique. 6-8, March.

MWANGWELA, A.M., WANISKA, R.D., PELEMBE, L. & MINNAAR, A. 2005. Value-added, processing and qualities of cowpea and bean-based foods. Conference on "Regional partenerships to enhance Bean/Cowpea consumption and production in Africa and Latin America". Dakar, Senegal. 12-16, September.

MWANGWELA, A.M., WANISKA, R.D. & MINNAAR, A. 2005. The quest for convenient, dried, whole cowpeas: Use of micronisation to reduce the cooking time of cowpeas (*Vigna unguiculata* L. Walp). Trend-Spotting 2005: Fashion-Driven Food Science, an18th South African Association of Food Science and Technology (SAAFoST) biennial international congress. Stellenbosch, South Africa. 5-8, September.

MWANGWELA, A.M., WANISKA, R.D., HALL, A. & MINNAAR, A. 2004. Structural changes during hydrothermal processing of cowpea seeds. Extended abstract published in the Proceedings the 43rd annual conference of the Microscopy Society of Southern Africa, Pretoria, South Africa.

Seminar presentations

MWANGWELA, A.M., WANISKA, R.D. & MINNAAR, A. The quest for convenient, dried, whole cowpeas: Use of micronisation to reduce the cooking time of cowpeas (*Vigna unguiculata* L. Walp). Presented to the inter-collegiate department of Food Science and Technology, Texas, A & M University, College Station, Texas. 1st April 2005.